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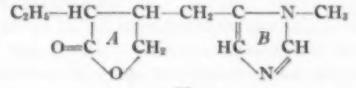
VOLUME XL | *FEBRUARY, 1951* | NUMBER 2 | CONSECUTIVE NO. 4

The Synthesis of Certain Amines Related to Pilocarpine*,†

By EINAR BROCHMANN-HANSSEN,‡ GLENN L. JENKINS, and JOHN B. DATA§

Certain tertiary derivatives of *dl*-isopilopylamine and *dl*-homoiospilopylamine have been prepared and evaluated pharmacologically for parasympathomimetic activity. Preparative methods used are described. Pharmacological studies indicate that dimethyl-*dl*-isopilopylamine and dimethyl-*dl*-homoisopilopylamine possess some parasympathomimetic action but the activity is considerably weaker than that of pilocarpine.

PILOCARPINE and isopilocarpine are two naturally occurring alkaloids which are represented by the structural formula I; these compounds differ only in stereochemical configuration (1, 2) and thus are two of the four possible stereoisomers having formula I. The naturally occurring forms are dextrorotatory. Pilocarpine is isomerized to isopilocarpine by heat alone or with alcoholic sodium hydroxide (3). Only pilocarpine is of importance medicinally for its parasympathomimetic action.



(I)

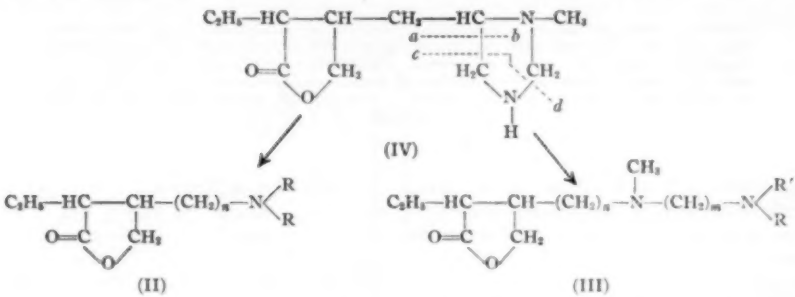
Examination of formula I reveals two cyclic structures; namely, the lactone ring *A*, an internal ester, and the imidazole ring *B*. These structures are found singly in other physiologically important substances. For example, the imidazole ring occurs in histamine and histadine, and the lactone ring occurs in ascorbic acid, cardiac glycosides, and santonin. It would be of interest to examine the specificity of ring *A* and *B* in pilocarpine relative to their importance for pilocarpine-like action.

There are certain indications in the literature that the intact lactone ring might be of significance. Jowett, Pyman, and Remfry (4) reported that the presence of an esterified carboxyl group in certain imidazole derivatives is not sufficient to confer pilocarpine-like action. Pyman (5) prepared several butyrolactone derivatives in which a 2-imidazolylmethyl group is attached to the *alpha* position of the lactone ring instead of the *beta* position as in pilocarpine. These compounds did not give pilocarpine-like action. Activity is lost if the lactone ring is opened by treating pilocarpine with alkali (6).

* Received May 5, 1950, from the Research Laboratories, Purdue University, School of Pharmacy, Lafayette, Ind.
Presented to the Scientific Section, A. PH. A., Atlantic City meeting, May, 1950.
† This work was made possible through the research fellowship established by Miles Laboratories, Inc., Elkhart, Ind.
‡ Purdue Research Foundation Fellow, 1946–1949. Present address: University of California, College of Pharmacy, San Francisco.
§ The authors wish to thank Dr. LeRoy D. Edwards for his advice and guidance in the pharmacological portion of this study.

There seem to be no indications in the literature that there is any relationship between the physiological properties of pilocarpine and those of other imidazole derivatives. The action of imidazole appears to be related closely to histamine (7); namely, musculotropic. Certain hydroxyalkyl and alkyl-substituted imidazoles increase the activity of imidazole or may change it qualitatively (8).

In light of these facts, it appeared of interest to prepare and evaluate pharmacologically certain compounds which are homologs of the general formulas II and III, in order to observe the effect which results from opening the imidazole ring while retaining the lactone ring intact. General formulas II and III are related to I as follows: hydrogenation of ring *B* in I gives the imidazolidine derivative IV. Rupture of the imidazolidine ring at line *ab* with simultaneous addition of two hydrogen atoms gives a compound of the general formula III in which *n* is 2, *m* is 1, R is CH_3—, and R′ is H. Rupture of the imidazolidine ring at line *cd* with simultaneous addition of four hydrogen atoms gives a compound of the general formula II in which *n* is 2, and R is CH_3—.



(II) (IV) (III)

Four amines of the general formula II and two diamines of the general formula III were synthesized. These compounds were prepared as represented on page 63.

dl-Pilopic acid (X) and *dl*-isopilopic acid (X) were prepared as follows: ethyl α-bromobutyrate (V) was allowed to react with diethyl malonate in the presence of sodium ethoxide, and the resultant product successively hydrolyzed, decarboxylated, and esterified to give diethyl ethylsuccinate (VI). Compound VI was allowed to react with ethyl formate in the presence of sodium ethoxide, and the resultant product acidified to give diethyl α-ethyl-β-formylsuccinate (VII). The formyl-ester was reduced with amalgamated aluminum in moist ether to diethyl α-ethyl-β-hydroxymethylsuccinate (VIII) which upon distillation split off ethyl alcohol to give ethyl α-ethylbutyrolactone-β-carboxylate (IX). This is a mixture of ethyl *dl*-pilopate and ethyl *dl*-isopilopate. The two forms were separated by fractional distillation, followed by recrystallization of the solid ester, ethyl *dl*-pilopate. Individual hydrolyses of the two forms gave *dl*-pilopic acid and *dl*-isopilopic acid.

N-Substituted *dl*-isopilopylamine hydrochlorides were prepared as follows: *dl*-isopilopoyl chloride (XI) was prepared from *dl*-isopilopic acid (X) and thionyl chloride, and the acid-chloride was reduced catalytically by the Rosenmund reduction method to *dl*-isopilopaldehyde (XII). Compound XII was reduced with amalgamated aluminum in moist ether to *dl*-isopilopyl alcohol (XIII), and the alcohol was converted to *dl*-isopilopyl bromide (XIV) with phosphorus tribromide. Compound XIV was condensed with dimethylamine, diethylamine, and N,N,N′-trimethylethylenediamine to give compounds of the general formula XV in which R,R′ is CH_3—, CH_3—, and C_2H_5—,C_2H_5—, and CH_3—, $(CH_3)_2NCH_2CH_2$—, respectively.

N-Substituted *dl*-homoisopilopylamine hydrochlorides were prepared as follows: *dl*-isopilopoyl chloride (XI) was converted by the Arndt-Eistert reaction to ethyl *dl*-homoisopilopate (XVI), and the ester was then hydrolyzed to *dl*-homoisopilopic acid (XVII). The acid was allowed to react with thionyl chloride to give *dl*-homoisopilopoyl chloride (XVIII). *dl*-Homoisopilopaldehyde (XIX) was prepared from XVIII by means of the Rosenmund reduction method, and the aldehyde was reduced with amalgamated aluminum in moist ether to *dl*-homoisopilopyl alcohol (XX). Compound XX was treated with phosphorus tribromide to give *dl*-homoisopilopyl bromide (XXI), and the latter compound was condensed with dimethylamine, diethylamine, and N,N,N′-trimethylethylenediamine to give compounds of the general formula XXII in which R,R′ is CH_3—,CH_3—, and C_2H_5,C_2H_5—, and CH_3—,$(CH_3)_2NCH_2CH_2$—, respectively.

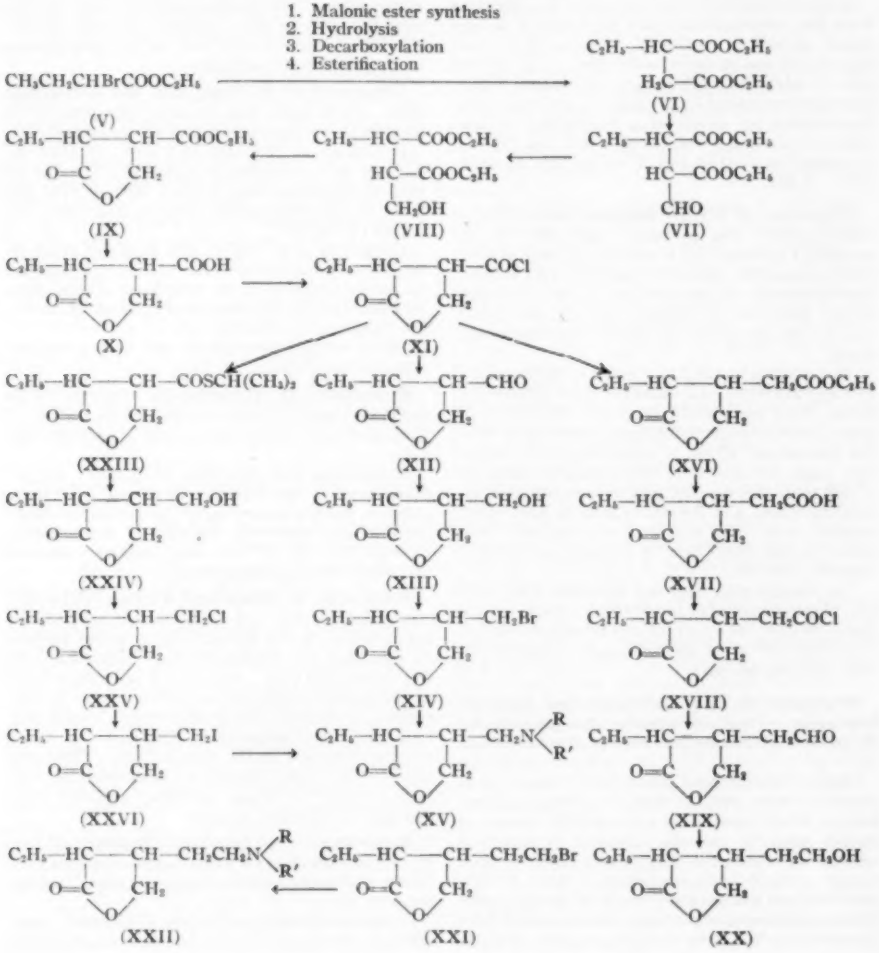
The following procedure was tried as a feasible approach for the preparation of N-substituted *dl*-pilopylamine hydrochlorides. *dl*-Pilopoyl chloride (XI) was condensed with 2-propanethiol to give compound XXIII. The latter compound was reduced catalytically with Raney nickel to the alcohol (XXIV). The resultant alcohol was converted to the iodide (XXVI) by way of the chloride (XXV). The condensation of compound XXVI with dimethylamine gave a compound which had the same melting point as dimethyl-*dl*-isopilopylamine hydrochloride (XV, R,R′ is CH_3—,CH_3—) and the mixed melting point was not depressed. From these results alone the indications are that isomerization had taken place at some stage of the synthesis.

All amines represented by formulas II and III prepared in this study were evaluated pharmacologically. The hydrochlorides of dimethyl-*dl*-isopilopylamine (XV, R,R′ is CH_3—,CH_3—) and dimethyl-*dl*-homoisopilopylamine (XXII, R,R′ is CH_3—,CH_3—) were found to possess some parasympathomimetic action similar to pilocarpine but considerably weaker.

EXPERIMENTAL

All melting points are uncorrected. Nitrogen was determined by a semimicro Kjeldahl method. Sulfur determinations were done by Huffmann Microanalytical Laboratories, Denver, Colo.

Preparation of N,N,N′-Trimethylethylenediamine.—N-Phenyl-N,N′,N′-trimethylethylenediamine was prepared according to the general procedure



described by Munch, Thannhauser, and Cottle (9) for α-dialkylamino-ω-methylanilinoalkanes.

N-Methylaniline, 321.5 Gm. (3.0 moles), was condensed with 144.0 Gm. (1.0 mole) of β-dimethylaminoethyl chloride hydrochloride to give 138.0 Gm. (77.5%) of product, b. p. 126–128° at 14 mm. pressure.

This product was converted to N,N,N′-trimethylethylenediamine dihydrochloride by means of nitrosation and hydrolysis of the nitroso derivative according to the general procedure described by the same authors. The amine was steam-distilled from the reaction mixture into a slight excess of hydrochloric acid solution (10%). The aqueous solution of N,N,N′-trimethylethylenediamine dihydrochloride was concentrated to dryness under reduced pressure, and the residue was recrystallized from absolute alcohol to give 98.5 Gm. (72.6%) of product, m. p. 187 to 187.5°.

Anal. Calcd. for $C_5H_{16}N_2Cl_2$: N, 16.00. Found: N, 15.86, 15.92.

N,N,N′-Trimethylethylenediamine was liberated from the dihydrochloride salt by means of a large excess of sodium hydroxide solution (50%), and the mixture was extracted with ether. The ethereal extract was dried with anhydrous sodium sulfate, the solvent removed by distillation, and the residue fractionated by means of a Podbielniak column (30–50 theoretical plates) to give 46.4 Gm. (80.9%) of product, b. p. 114.5 to 115° at 750 mm. pressure; $n_D^{25} = 1.420$.

Preparation of Diethyl Ethylsuccinate.—Diethyl ethylsuccinate was prepared essentially by the method of Carriere (10) in an over-all yield of 72%. The condensation of diethyl malonate with ethyl α-bromobutyrate was modified according to the procedure described by Gyngell, Phillips, and Smith (11) for the preparation of diethyl ethylphenylmalonate.

To a solution of 160.0 (1.0 mole) of diethyl malonate and 215.0 Gm. (1.1 moles) of ethyl α-bromobutyrate, there was added a solution of 25.0 Gm. (1.08 gram atoms) of sodium dissolved in 450 ml. of absolute alcohol and 10 ml. of anhydrous ethyl acetate. The crude triethyl 1,1,2-butanetricarboxylate obtained from this reaction was hydrolyzed with hydrochloric acid and decarboxylated to give ethylsuccinic acid. The acid was recrystallized from water to give 126.0 Gm. of product, m. p. 100°; reported (10), 98°.

The ethylsuccinic acid was esterified with 120.0 Gm. of absolute alcohol and 64.0 Gm. of sulfuric acid (98%) to give 146.0 Gm. of diethyl ethylsuccinate, b. p. 110–113° at 14 mm. pressure; reported (2), 105° at 9 mm. pressure.

Preparation of Ethyl *dl*-Pilopate and Ethyl *dl*-Isopilopate.—These compounds were prepared by the method of Tschitschibabin and Preobrashenski (2).

Diethyl ethylsuccinate, 202.0 Gm. (1.0 mole), was allowed to react with 81.5 Gm. (1.1 moles) of ethyl formate in the presence of a theoretical amount of sodium ethoxide, and the resultant product was acidified with sufficient sulfuric acid (10%) to give diethyl α-ethyl-β-formylsuccinate. The formylester was reduced with an excess of amalgamated aluminum prepared according to the method of Wislicenus (12). When the reduction was complete the reaction mixture was filtered and the ether was removed by distillation. The residue, consisting of diethyl α-ethyl-β-hydroxymethylsuccinate, was distilled under reduced pressure. Ethyl alcohol was split off in the distillation process, and the resulting lactone, ethyl α-ethylbutyrolactone-β-carboxylate, distilled at 146–152° at 14 mm. pressure; reported (2), 142–152° at 9 mm. pressure. The yield was 128.0 Gm. (68.8%).

Several runs were pooled and the product was fractionated carefully under reduced pressure using a modified Claisen distilling head. Fraction no. 1 (110.0 Gm.) distilled at 150–152° at 14 mm. pressure, fraction no. 2 (24.0 Gm.) distilled at 152–155° at 14 mm. pressure, and fraction no. 3 (98.0 Gm.) distilled at 155–157° at 14 mm. pressure. These fractions were placed in a refrigerator, and after approximately one month fraction no. 3 crystallized to a solid mass. Fraction no. 2 was seeded and after a few days about one-half crystallized. The crystals were collected and recrystallized from absolute ether. The melting point of ethyl *dl*-pilopate was 48.5 to 49°; reported (2), 48.5 to 49°. The liquid fraction no. 1 and the oil from fraction no. 2 was ethyl *dl*-isopilopate.

Preparation of *dl*-Pilopic Acid and *dl*-Isopilopic Acid.—These acids were prepared according to the method of Tschitschibabin and Preobrashenski (2).

Hydrolysis of 18.6 Gm. (0.1 mole) of ethyl *dl*-pilopate with hydrochloric acid (12%) followed by recrystallization of the acid from benzene gave 14.4 Gm. (91.1%) of *dl*-pilopic acid, m. p. 90–91°; reported (2), 90–91°.

Hydrolysis of 37.2 Gm. (0.2 mole) of ethyl *dl*-isopilopate with hydrochloric acid (12%) followed by recrystallization of the acid from benzene gave 30.4 Gm. (96.2%) of *dl*-isopilopic acid, m. p. 87.5 to 88°; reported (2), 87.5 to 88°.

Mixed melting point of the two acids gave considerable depression.

Preparation of *dl*-Isopilopoyl Chloride.—This compound was prepared according to the method of Preobrashenski, Poljakowa, and Preobrashenski (13).

dl-Isopilopic acid, 30.0 Gm. (0.19 mole), was allowed to react with 68.0 Gm. (0.57 mole) of thionyl chloride, purified according to the method of Fieser (14), to give 32.3 Gm. (96.1%) of *dl*-isopilopoyl chloride, b. p. 115–117° at 1 mm. pressure; reported (13), 90.2° at 0.05 mm. pressure.

Preparation of *dl*-Isopilopyl Alcohol.—*dl*-Isopilopoyl chloride, 32.3 Gm. (0.18 mole), was reduced catalytically by the Rosenmund reduction method as described by Preobrashenski, Poljakowa, and Preobrashenski (13). Removal of the solvent used in the reaction under reduced pressure gave an oily residue of impure *dl*-isopilopaldehyde, which was reduced with amalgamated aluminum in moist ether as described by the same authors. There was obtained 19.5 Gm. (74.4%) of *dl*-isopilopyl alcohol, b. p. 132–135° at 2 mm. pressure; reported (13), 116–117° at 0.07 mm. pressure.

Preparation of *dl*-Isopilopyl Bromide.—*dl*-Isopilopyl bromide was prepared according to the procedure of Preobrashenski, Poljakowa, and Preobrashenski (13).

dl-Isopilopyl alcohol, 19.5 Gm. (0.135 mole), was allowed to react with 13.5 Gm. (0.05 mole) of freshly

distilled phosphorus tribromide to give 20.7 Gm. (74.1%) of *dl*-isopilopyl bromide, b. p. 122–124° at 2 mm. pressure; reported (13), 91° at 0.06 mm. pressure.

Preparation of Dimethyl-*dl*-isopilopylamine Hydrochloride.—A solution of 6.9 Gm. (0.033 mole) of *dl*-isopilopyl bromide in 20 ml. of dry xylene was cooled in a dry ice–acetone bath; then 3.1 Gm. (0.069 mole) of cold, anhydrous dimethylamine was added, and the mixture was sealed in a glass tube. The tube was shaken occasionally during forty-eight hours at room temperature and then opened. The crystals consisting primarily of dimethylamine hydrobromide were filtered off, and the xylene and excess dimethylamine were removed by distillation under reduced pressure. The residue was dissolved in anhydrous ether, the solution was filtered, and the dimethyl-*dl*-isopilopylamine was precipitated from the solution as the hydrochloride by the addition of dry hydrogen chloride. The salt was recrystallized from absolute alcohol to give 2.2 Gm. (32.3%) of product, m. p. 205 to 205.5°.

Anal. Calcd. for $C_8H_{18}NO_2Cl$: N, 6.74. Found: N, 6.69, 6.76.

Preparation of Diethyl-*dl*-isopilopylamine Hydrochloride.—A solution of 6.9 Gm. (0.033 mole) of *dl*-isopilopyl bromide, 5.0 Gm. (0.069 mole) of diethylamine, and 20 ml. of dry xylene was heated at 140–150° for ten hours in an oil bath. The product was worked up as described above for dimethyl-*dl*-isopilopylamine hydrochloride. The hydrochloride salt was recrystallized from absolute alcohol to give 3.1 Gm. (40.0%) of product, m. p. 174–175°.

Anal. Calcd. for $C_{11}H_{22}NO_2Cl$: N, 5.94. Found: N, 6.00, 6.06.

Preparation of N-*dl*-Isopilopyl-N,N′,N′-trimethylethylenediamine Dihydrochloride.—This compound was prepared by the procedure described above for diethyl-*dl*-isopilopylamine hydrochloride.

dl-Isopilopyl bromide, 6.9 Gm. (0.033 mole), was condensed with 7.0 Gm. (0.069 mole) of N,N,N′-trimethylethylenediamine. The dihydrochloride salt was recrystallized from alcohol (95%) to give 4.7 Gm. (47.4%) of product, m. p. 227 to 227.5°.

Anal. Calcd. for $C_{12}H_{28}N_2O_2Cl_2$: N, 9.30. Found: N, 9.15, 9.20.

Preparation of Ethyl *dl*-Homoisopilopate.—This compound was prepared from *dl*-isopilopoyl chloride by the Arndt-Eistert synthesis essentially as described by Poljakowa, Preobrashenski, and Preobrashenski (15).

A solution of 35.3 Gm. (0.2 mole) of *dl*-isopilopoyl chloride in 100 ml. of anhydrous ether was added slowly to an ethereal solution of diazomethane while maintaining the temperature of the reaction mixture between −5 to 0°. The ethereal solution of diazomethane was prepared from 90.0 Gm. of nitrosomethylurea according to the method described by Bachmann and Struve (16). After allowing the reaction mixture to stand for twenty-four hours in an ice chest, the solvent was removed under reduced pressure at 20–30°. The residue was dissolved in 200 ml. of absolute alcohol. The solution was heated to 55–60° and then treated with 3.6 Gm. of silver oxide suspended in alcohol in the usual way. The reaction mixture was filtered, the alcohol was removed under reduced pressure, and the residue was fractionated to give 30.1 Gm. (75.2%) of product, b. p. 128–130° at 2 mm. pressure; reported (15)' b. p. 129–130° at 2.5 mm. pressure.

Preparation of *dl*-Homoisopilopic Acid.—This acid was prepared according to the method of Poljakowa, Preobrashenski, and Preobrashenski (15).

Hydrolysis of 30.0 Gm. (0.15 mole) of ethyl *dl*-homoisopilopate with hydrochloric acid (12%) followed by recrystallization of the acid from absolute alcohol gave 23.5 Gm. (91.1%) of *dl*-homoisopilopic acid, m. p. 74.5°; reported (15), 74.2°.

Preparation of *dl*-Homoisopilopoyl Chloride.—This compound was prepared according to the method described above for *dl*-isopilopoyl chloride.

dl-Homoisopilopic acid, 23.0 Gm. (0.13 mole), was allowed to react with 46.3 Gm. (0.39 mole) of purified thionyl chloride to give 22.7 Gm. (89.7%) of *dl*-homoisopilopoyl chloride, b. p. 125–127° at 2 mm. pressure; reported (17), 160–162° at 10 mm. pressure.

Preparation of *dl*-Homoisopilopyl Alcohol.—This compound was prepared according to the procedure described above for *dl*-isopilopyl alcohol.

dl-Homoisopilopoyl chloride, 22.7 Gm. (0.12 mole), was reduced catalytically by the Rosenmund reduction method to *dl*-homoisopilopaldehyde. The aldehyde was reduced with amalgamated aluminum in moist ether to give 12.2 Gm. (64.9%) of *dl*-homoisopilopyl alcohol, b. p. 160–161° at 3 mm. pressure; reported (17), 161–163° at 3 mm. pressure.

Preparation of *dl*-Homoisopilopyl Bromide.—This compound was prepared according to the method described above for *dl*-isopilopyl bromide.

dl-Homoisopilopyl alcohol, 12.2 Gm. (0.08 mole), was allowed to react with 7.7 Gm. (0.03 mole) of freshly distilled phosphorus tribromide to give 11.9 Gm. (70.0%) of *dl*-homoisopilopyl bromide, b. p. 125–127° at 2 mm. pressure; reported (17), 160° at 8 mm. pressure.

Preparation of Dimethyl-*dl*-homoisopilopylamine Hydrochloride.—This compound was prepared from dimethylamine and *dl*-homoisopilopyl bromide according to the procedure described above for dimethyl-*dl*-isopilopylamine hydrochloride.

dl-Homoisopilopyl bromide, 4.4 Gm. (0.02 mole), was allowed to react with 1.8 Gm. (0.04 mole) of dimethylamine. The hydrochloride salt was recrystallized from absolute alcohol to give 1.3 Gm. (29.5%) of dimethyl-*dl*-homoisopilopylamine hydrochloride, m. p. 170 to 170.5°.

Anal. Calcd. for $C_{10}H_{30}NO_2Cl$: N, 6.32. Found: 6.20, 6.25.

Preparation of Diethyl-*dl*-homoisopilopylamine Hydrochloride.—This compound was prepared from diethylamine and *dl*-homoisopilopyl bromide according to the procedure described above for diethyl-*dl*-isopilopylamine hydrochloride.

dl-Homoisopilopyl bromide, 4.4 Gm. (0.02 mole), was allowed to react with 2.9 Gm. (0.04 mole) of diethylamine. The hydrochloride salt was recrystallized from absolute alcohol to give 1.2 Gm. (24.1%) of diethyl-*dl*-homoisopilopylamine hydrochloride, m. p. 147°.

Anal. Calcd. for $C_{12}H_{24}NO_2Cl$: N, 5.61. Found: N, 5.74, 5.78.

Preparation of N-*dl*-Homoisopilopyl-N,N′,N′-trimethylethylenediamine Dihydrochloride.—This compound was prepared according to the method de-

scribed above for N-*dl*-isopilopyl-N,N',N'-trimethylethylenediamine dihydrochloride.

dl-Homoisopilopyl bromide, 3.1 Gm. (0.014 mole), was allowed to react with 2.6 Gm. (0.028 mole) of N,N,N'-trimethylethylenediamine. The dihydrochloride salt was recrystallized from alcohol (95%) to give 1.1 Gm. (25.0%) of product, m. p. 180–181°.

Anal. Calcd. for $C_{13}H_{28}N_2O_2Cl_2$: N, 8.89. Found: N, 8.82, 8.85.

Attempt to Prepare Dimethyl-*dl*-pilopylamine Hydrochloride from *dl*-Pilopic Acid.—*dl*-Pilopic acid was converted to *dl*-pilopoyl chloride with thionyl chloride according to the procedure of Preobrashenski, Poljakowa, and Preobrashenski (18). These authors have shown that this method does not cause isomerization.

A mixture of 14.2 Gm. (0.09 mole) of *dl*-pilopic acid and 53.0 Gm. (0.45 mole) of purified thionyl chloride was heated at 40–45° for five hours and then at 75–80° for thirty minutes. The excess thionyl chloride was removed by distillation under reduced pressure (12–15 mm.). The last traces of thionyl chloride were removed by addition of five 10-ml. portions of anhydrous benzene and removal of the benzene by distillation under reduced pressure after each 10-ml. addition. The residual *dl*-pilopoyl chloride was not distilled, but the impure product was used in the next step.

The residual *dl*-pilopoyl chloride was dissolved in 50 ml. of dry benzene, and to this solution there was added 34.0 Gm. (0.45 mole) of 2-propanethiol and 7.1 Gm. (0.09 mole) of dry, freshly distilled pyridine. After forty-eight hours at room temperature, 50 ml. of water and 50 ml. of ether were added, and the mixture was shaken in a separatory funnel. The organic layer was collected and shaken twice with a small volume of sulfuric acid (1%) and then once with water. The organic solution was dried over anhydrous sodium sulfate, and the solvent and excess 2-propanethiol were removed under reduced pressure (12–15 mm.). The crystalline residue was recrystallized from absolute ether. The thioester XXIII melted at 63°. The yield was 18.1 Gm.

Anal. Calcd. for $C_{10}H_{16}O_2S$: S, 14.83. Found: S, 14.85, 14.79.

The thioester was reduced according to the method of Jeger, Norymberski, Szpilfogel, and Prelog (19) for the conversion of acid-chlorides to alcohols by catalytic reduction of thioesters under mild conditions.

The thioester XXIII, 7.6 Gm. (0.035 mole), was dissolved in 50 ml. of alcohol (95%) and then added to 75 Gm. of Raney nickel catalyst in 150 ml. of alcohol (95%). The catalyst was prepared according to the procedure of Mozingo, Wolf, Harris, and Folkers (20). The mixture was shaken mechanically for two hours, and then the catalyst was centrifuged off and washed several times with alcohol. Concentration of the alcoholic solution under reduced pressure (12–15 mm.) gave 4.6 Gm. of a liquid residue XXIV.

The residual product XXIV was then treated successively with thionyl chloride and sodium iodide according to the method of Preobrashenski, Poljakowa, and Preobrashenski (18) for the conversion of *dl*-pilopyl alcohol to *dl*-pilopyl iodide. According to these authors, this method does not cause isomerization.

The liquid residue XXIV was allowed to react with 4.2 Gm. (0.035 mole) of thionyl chloride. Removal of the thionyl chloride under reduced pressure gave 5.0 Gm. of an oily residue XXV which was used without further purification in the next step. The residue XXV was allowed to react with 4.6 Gm. (0.031 mole) of anhydrous sodium iodide in 33 ml. of acetone. Removal of the solvent gave 5.5 Gm. of an oily residue XXVI, which was used without further purification in the next step.

The oily residue XXVI was allowed to react with dimethylamine according to the procedure described above for dimethyl-*dl*-isopilopylamine hydrochloride. The hydrochloride obtained from this condensation melted at 205 to 205.5°, and when mixed with dimethyl-*dl*-isopilopylamine hydrochloride did not depress the melting point.

From these results alone the indications are that isomerization had taken place at some stage of the synthesis, and that the intermediate products which were obtained are as represented, but their configurations are uncertain.

PHARMACOLOGY

After the first few experiments using the excised rabbit intestine, it became obvious that only two of the compounds prepared in this study might possibly possess parasympathomimetic action; namely, dimethyl-*dl*-isopilopylamine hydrochloride and dimethyl-*dl*-homoisopilopylamine hydrochloride. Therefore, in most of the experiments only these two compounds were tested. The pharmacological actions of these compounds were compared to pilocarpine hydrochloride under comparable conditions.

The Action on the Excised Rabbit Intestine.—The Magnus method (21) was used for recording the movements of the excised intestinal ileum. Dimethyl-*dl*-isopilopylamine hydrochloride and dimethyl-*al*-homoisopilopylamine hydrochloride produced a considerable increase in contractions but only a slight increase in tonus. After atropine had been added, these compounds had no effect. The other amines synthesized in this study had a slight depressant action on the gut.

The Action on the Blood Pressure of the Intact Cat.—Healthy, nonpregnant cats weighing 2–4 Kg. were used. The cats were anesthetized by intraperitoneal injection of 1 ml./Kg. of a solution of sodium pentobarbital (3%) and urethane (25%). Dimethyl-*dl*-isopilopylamine hydrochloride and dimethyl-*dl*-homoisopilopylamine hydrochloride produced a transient fall in blood pressure similar to, but much less pronounced, than that produced by pilocarpine. Atropine inhibited this effect on the blood pressure.

Perfusion of the Frog's Heart *in situ*.—The procedure used was that described by Sollmann and Hanzlik (22). Qualitatively, dimethyl-*dl*-isopilopylamine hydrochloride and dimethyl-*dl*-homoisopilopylamine hydrochloride showed the same type of action as pilocarpine both before and after paralysis of the vagus.

The Action on the Pupil of the Cat's Eye.—The application of dimethyl-*dl*-homoisopilopylamine hydrochloride in doses of $1^1/_4$ and $12^1/_2$ mg. caused no change in the size of the pupil of a cat's eye. It is interesting to point out that isopilocarpine does not seem to have any marked effect on the pupil, either.

However, this may, as Dixon and Ranson (23) have pointed out, be due to the fact that very concentrated solutions must be applied because of the low potency. These solutions are only absorbed slowly and are soon diluted or washed away by tears.

The Action on the Salivary and Lacrimatory Glands of Rats.—Intraperitoneal injection of 0.6 Gm./Kg. of dimethyl-*dl*-isopilopylamine hydrochloride in rats produced salivation, lacrimation, and defecation to an extent approximately equal to the effect produced by 20 mg./Kg. of pilocarpine hydrochloride.

Test for Antispasmodic Activity.—Diethyl-*dl*-isopilopylamine hydrochloride and N-*dl*-isopilopyl-N,N′,N′-trimethylethylenediamine dihydrochloride were tested for antispasmodic activity according to the method of Graham (24). These compounds had practically no activity against spasms produced by barium chloride. The activity against spasm produced by Mecholyl was slightly greater, but of such low magnitude as to be of no significance.

Conclusions.—Pharmacological studies indicate that dimethyl-*dl*-isopilopylamine hydrochloride and dimethyl-*dl*-homoisopilopylamine hydrochloride possess some parasympathomimetic action. There is not much difference in their potency. The activity is considerably weaker than that of pilocarpine.

The pharmacological properties of these two compounds support further the indications that the intact lactone ring *A* in I appears to be of some importance for pilocarpine-like action. These results indicate also that the basic imidazole ring *B* in I appears to be less specific, since in this study the imidazole ring has been substituted by a dimethylamino group without totally destroying pilocarpine-like action.

SUMMARY

1. Six amine hydrochlorides have been prepared and evaluated pharmacologically. These are: dimethyl-*dl*-isopilopylamine; diethyl-*dl*-isopilopylamine; N-*dl*-isopilopyl-N,N′,N′-trimethylethylenediamine; dimethyl-*dl*-homoisopilopylamine; diethyl-*dl*-homoisopilopylamine; and N-*dl*-homoisopilopyl-N,N′,N′-trimethylethylenediamine hydrochlorides.

2. Pharmacological studies indicate that dimethyl-*dl*-isopilopylamine and dimethyl-*dl*-homoisopilopylamine hydrochloride possess some parasympathomimetic action, but the activity is considerably weaker than that of pilocarpine.

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Ascaridole in Chenopodium Oil. IV. The Synthesis of Ascaridole*

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Ascaridole, the active ingredient of chenopodium oil, was synthesized from terpediene. The physical and chemical properties of the reaction product and its intermediates are presented. The reaction is discussed as a possible means of biosynthesis of this compound in the plant.

THE reported (1) existence of ascaridole as the only naturally occurring transannular peroxide is both intriguing and challenging when consideration is given to the rather widespread presence of α-terpinene, its postulated precursor. While it is rather well known that ascaridole is the only therapeutically active ingredient of chenopodium oil and its structural chemistry has been given rather close attention (2, 3), few attempts have been made at its synthesis.

Bodendorf (4) reported the isolation of ascaridole from auto-oxidized α-terpinene. Elson, Gibson, and Simonsen (5) attempted to synthesize ascaridole by treating α-terpinene with benzoylhydroperoxide, but were unsuccessful. The chief reaction product other than *p*-cymene (30 per cent) was 1,4-oxido-Δ^2-*p*-menthene, which was proved by oxidation to 1,4-cineolic acid. It is important to note that ascaridole may also be degraded to 1,4-cineolic acid. Recently a synthesis of ascaridole was reported (6) but no synthetic details nor structural proof were given. A patent (7) also described the synthesis of "endoperoxides of the ascaridole type" but here, too, no structural proof was offered. A difficulty that is present in the characterization of ascaridole is the notable lack of a solid derivative. While rearranged products may be obtained fairly easily and with some degree of purity, the physical constants of the oil remain as the prime determinants. Even the determination of the peroxide activity of the compound is open to some question (8).

Recently the properties of ascaridole, obtained with a high degree of purity, from chenopodium oil were reported (9). It became of interest to attempt to prepare the synthetic product and compare its properties with the naturally occurring one.

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EXPERIMENTAL

Preparation of α-Terpinene

Cardamom oil was distilled and the fraction boiling at 170–210° was collected. This fraction was dissolved in an equal weight of ether (dried over sodium) and cooled to 0° with an ice-salt mixture. Dry hydrogen chloride was passed through the cold mixture until no further gain in weight was observed. The mixture was removed from the ice bath and allowed to stand at room temperature for two hours. The solvent was evaporated and the solid residue (m. p. 43–48°) distilled under diminished pressure and recrystallized from absolute ethanol, to yield terpinene dihydrochloride, b. p. 106–108°/1–2 mm. pressure; m. p. 50–51°.

Terpinene dihydrochloride, 20 Gm., was warmed with 40 cc. of freshly distilled aniline until the reaction started. After twenty minutes, 40 cc. of glacial acetic acid was added and the mixture steam-distilled. The distillate was shaken with 10% oxalic acid solution and the steam distillation repeated. The distillate was acidified with dilute sulfuric acid and extracted with ether. The ethereal extracts were washed with water, dried over anhydrous sodium sulfate, and the solvent evaporated. The residue was distilled under reduced pressure to yield α-terpinene: b. p. 175–177°; d_{20}^{20} 0.8484; n_D^{20} 1.4767.

This product was further identified by forming the maleic anhydride adduct through the Diels-Alder reaction. A 10-Gm. sample of the α-terpinene was refluxed with 7 Gm. of maleic anhydride in 25 cc. of ether for one hour. The ether was evaporated and the residue recrystallized from methanol m. p. 63–65°; reported m. p., 64–65° (10).

Tishchenko and Bogomolov (11) reported that the dry distillation of the barium salt of the maleic anhydride adduct would regenerate α-terpinene. Since γ-terpinene could be suspected in a product derived from natural sources, and, also, since only α-terpinene could take part in the Diels-Alder reaction, further purification was attempted in the manner suggested by them (11).

The maleic anhydride adduct was formed from 50 Gm. of α-terpinene (obtained from the dihydrochloride in the manner described above) and 25 Gm. of maleic anhydride in 150 cc. of ether. The ether was evaporated and 500 cc. of 0.1 *N* NaOH added, the mixture heated to boiling, cooled, and acidified with dilute hydrochloric acid. The copious precipitate was filtered, washed with cold water, dried, and recrystallized from ethanol. The recrystallized product melted at 126–128°. The reported melting point of the acid is 127–128° (10).

The barium derivative was formed from a solution of the sodium salt and barium chloride and, after filtering, washing, and drying, was destructively distilled. The distillation did not proceed as smoothly as reported, and there was evidence of extensive degradation. The product was frac-

TABLE I.—PHYSICAL CONSTANTS OF THE FRACTIONATED OXIDIZED α-TERPINENE

Fraction	B. p., 1–2 Mm.	Weight, Gm.	%[a]	d_{20}^{20}	n_D^{20}	Peroxide Content,[b] %
A	Up to 65°	7.92	33	0.8644	1.4806	8
B	65–70°	6.72	28	0.9276	1.4746	18
C	70–75°	3.04	12.5	1.0021	1.4726	46
D	75–85°	2.58	10.7	1.0034	1.4730	61
E	Residue	3.00	12.5	1.0240	[c]	11

[a] Based upon a total charge of 24 Gm. of α-terpinene.
[b] By the method for ascaridole in chenopodium oil described in N. F. VIII. The equivalent factor for ascaridole was used.
[c] The material was too darkly colored to be used.

tionally distilled and the constant boiling portion at 178–180° was collected and redistilled twice, a middle cut being taken each time. The α-terpinene obtained in this manner had the following properties: b. p. 178–180°; n_D^{20} 1.4784; d_{20}^{20} 0.8463. The nitrosite derivative, prepared in the usual manner from a glacial acetic acid solution of the α-terpinene, melted at 155–156°; reported melting point of the nitrosite, 155° (3). The average yield of the purified α-terpinene was approximately 45%, with a range of 33–57% in five repeated trials.

The Preparation of Ascaridole

While α-terpinene is described as the most stable of the terpedienes (12), the ease with which these compounds may be oxidized is well known (13, 14).

In a pair of specially prepared[1] Vitreosil tubes (10 x 3.5 cm.), fitted with a thermometer and gas inlet and outlet tubes, was placed a solution of 2 Gm. of α-terpinene in 25 cc. of absolute ethanol and 5 cc. of benzene. The gas inlet tube was connected to an oxygen source and the gas was permitted to flow at a rate sufficient to cause gentle agitation. The reaction tubes were illuminated by the direct ultraviolet rays of a mercury-vapor lamp. Mirrors were placed about the apparatus to reflect the radiations so as to provide an even illumination of the reaction media. There was no apparent temperature change throughout the reaction period and no control of temperature was attempted.

After six hours of such treatment, the contents of both tubes were emptied into a boiling flask and the solvent evaporated at reduced pressure. The residual oil was distilled at 1–2 mm. pressure through a 10-in. glass-helices-packed column. Three crude fractions were obtained: fraction A, boiling up to 65°; fraction B, distilling at 65–80°; and fraction C, boiling at 80–105°. There was a small residue in the distilling flask which was plastic at room temperature.

The various fractions were assayed for peroxide activity and the following results were obtained: fraction A, 6%; fraction B, 18–20%; and fraction C, 30–40%. The residue showed no peroxide activity.

The synthesis was repeated, in the above manner, in order to obtain sufficient material to chemically identify the resultant products. The runs were successive and the material was stored in an ice chest until worked up. The physical constants of more closely fractionated materials resulting from the admixture of six runs, and representing a total charge of 24 Gm. of α-terpinene, are reported in Table I.

[1] Thermal Syndicate, Ltd., New York, N. Y.

The Identification of the Fractions

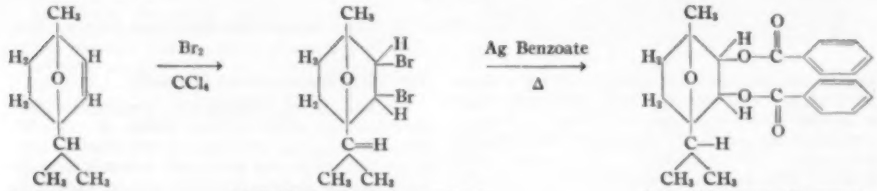
Fraction A was redistilled at atmospheric pressure and the major portion boiled at 175–178°. Since the physical constants of this fraction compared favorably with *p*-cymene, which is a major portion of chenopodium oil and was reported to be a by-product of the oxidation reaction (5), its presence was determined by oxidation with potassium permanganate. *p*-Hydroxybenzoic acid was isolated in a 53% yield, m. p. 154–155°.

Fraction B was also purified through distillation and the cut boiling at 112–115°/45 mm. was collected (86% of the original fraction) and had the following properties: d_{20}^{20} 0.9280; n_D^{20} 1.7426. A sample readily decolorized bromine water, and an attempt was made to form a bromine derivative.

A carbon tetrachloride solution of bromine was added dropwise to a solution of 1.35 Gm. of the redistilled fraction B in 20 cc. of the same solvent, until a permanent pink color was obtained. The solution was placed in an ice chest to crystallize. After two days, when no crystals were apparent the solvent was removed under reduced pressure, and an amber-colored oil, with a sharp odor of bromine, was obtained. This residue was dissolved in toluene, and 3 Gm. of silver benzoate was added. The mixture was refluxed for ten hours and the solvent evaporated. The residue was recrystallized from ethanol, m. p. 115–117°. This was shown to be ascaridole-glycol dibenzoate through the mixed melting point technique and through oxidation to 1,4-cineolic acid.

A sample of the dibenzoate, obtained from the previous reaction, was boiled with 25 cc. of a 5% NaOH solution for one-half hour. The cooled solution was extracted with three 10-cc. portions of ether and the solvent evaporated. The residue was mixed with 20 cc. of a 5% Na_2CO_3 solution and cooled to about 0° with small pieces of solid carbon dioxide which served as a refrigerant, an agitating device, and also to provide a neutral atmosphere. A 1% solution of potassium permanganate was added dropwise until a permanent pink color was maintained. The mixture was allowed to set at room temperature for two hours and then evaporated to dryness. The residual salts were extracted with chloroform and dissolved in the least amount of water, acidified with dilute HCl, and extracted with ether. The ether extract was dried and evaporated. The residue was crystallized from water, dried, and evaporated. The residue was crystallized from water and dried in a desiccator. The product melted at 120–122°. The reported melting point for 1,4-cineolic acid, obtained by oxidizing 1,4-oxido-Δ^2-*p*-menthene, is 121–122° (5).

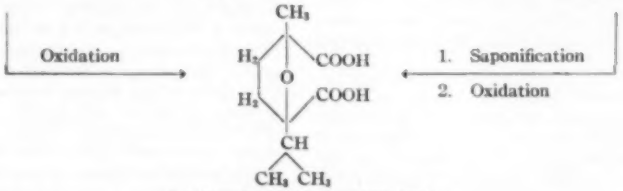
To further establish that the principal product of fraction B was 1,4-oxido-Δ^3-*p*-menthene, a sample of the redistilled fraction was oxidized in the above manner. The resultant product melted at 120–121° and was identical with the 1,4-cineolic acid obtained from the oxidation of the glycol (mixed melting point). The course of these reactions may be indicated as follows:



1,4-Oxido-Δ^3-*p*-menthene

1,4-Oxido-2,3-dibromomenthane (oil)

Ascaridol-glycol dibenzoate, m. p. 115–116°



1,4-Cineolic acid, m. p. 120–121°

The redistilled fraction B was tested for peroxide activity and showed an ascaridole content of 2–4%, which was probably due to contaminants present.

The redistilled, constant boiling cuts of both fractions C and D showed similar physical properties. About 88–93% of the combined material boiled at 73–76°/2 mm., d_{20}^{20} 1.0045, n_D^{20} 1.4720. The molecular refractivity (M_D) was calculated for ascaridole as 46.8; found 46.6. The freezing point of the sample was 3°. These constants are in excellent agreement with those found for a highly purified, naturally occurring ascaridole. A 0.254-Gm. sample assayed as 91% ascaridole by the iodine method.

To establish conclusively this material as ascaridole, it was rearranged to ascaridole-glycol anhydride with ferrous sulfate solution, and the formation of the benzoate derivatives resulted.

The Rearrangement of Ascaridole

Approximately 4 Gm. of the combined, redistilled fractions C and D was shaken with 25 cc. of a saturated solution of ferrous sulfate. The temperature of the reaction was kept at 5–10° with an ice bath. The color of the oily material changed rapidly and after one-half hour the material was extracted with ether. The ether was evaporated and the residue mixed with 3 Gm. benzoyl chloride and 25 cc. of 2% NaOH solution. A crystalline solid separated after a few minutes, and was filtered and recrystallized from absolute ethanol, m. p. 135–136°; reported m. p. for ascaridol-glycol monobenzoate, 136–137°. The ester was saponified with 2 *N* KOH, and the C_6H_5CO content of a weighed sample calculated.

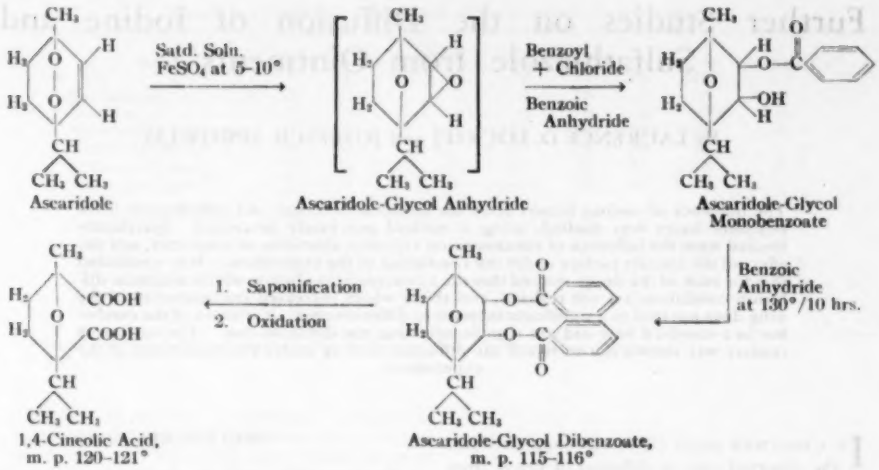
Anal.—Calcd. for $C_6H_5CO—C_{10}H_{17}O_3$: 36.21%. Found: 35.93%.

The dibenzoate was prepared by heating a sample of the monobenzoic acid ester with benzoic anhydride in 10 cc. of *p*-cymene for two hours at 130°. The solvent was evaporated under reduced pressure and the residue crystallized from ethanol; m. p. 116–117° (reported melting point for the dibenzoate derivative, 116.5 to 117.5°). The dibenzoate was hydrolyzed with 5% NaOH and oxidized with alkaline permanganate in the manner described above to yield 1,4-cineolic acid. The chemical behavior of ascaridole, prepared synthetically, may be indicated as shown in the equation on page 71.

Attempted redistillation on a small sample of fraction E resulted in extensive decomposition above 150°/1–2 mm. and no distillate was obtained. The entire fraction was dissolved in absolute ethanol and crystallized out by means of a solid carbon dioxide-acetone bath. After three such crystallizations, a light amber, resinous, plastic material was obtained that hardened to a glass at 0–10°. A definite melting point could not be obtained although the material liquefied at 40–45°. The purified material had the following physical properties: d_{20}^{20} 1.0251; n_D^{45} 1.4738.

A 0.255-Gm. sample showed practically no ascaridole present (2–3%). Since the oxidation of *α*-phellandrene with oxygen produced the ketone 4-isopropyl-Δ^2-hexene-1-one, a sample of fraction E was treated with 2,4-dinitrophenylhydrazine, and a small amount of a solid crystalline substance was obtained. On recrystallization from ethanol, this compound melted at 141–142°. Attempts at hydrolyzing the 2,4-dinitrophenylhydrazone to regenerate the ketone were unsuccessful. The usual group classification reagents failed to indicate the presence of any other functional groups. Fraction E probably consisted of the polymerized material described as resulting from the distillation of ascaridole and a by-product ketonic compound.

The synthesis of ascaridole from *α*-terpinene appears to proceed smoothly under the conditions noted. *α*-Terpinene has been reported to be present, in small amounts in chenopodium oil (3). On the basis of the course of the synthetic reaction, it may be regarded as the precursor for ascaridole in its



biogenesis in the plant. Similarly, the formation of *p*-cymene as a by-product of the reaction is also consistent with its presence in whole chenopodium oil. It is of interest to note that, while the synthetic process produced 1,4-oxido-Δ^2-*p*-menthene, its presence in natural chenopodium oil has not been established. This notable absence might be explained by the presence of a system that preferentially catalyzes the production of ascaridole from α-terpinene. Further work is in progress investigating the kinetics of the oxidation, and studying the influence of the plant on the course of the reaction.

SUMMARY

Ascaridole was synthesized through the photooxidation of α-terpinene. 1,4-Oxido-Δ^2-*p*-menthene, *p*-cymene, and a polymeric fraction were isolated as by-products of the reaction. An unidentified ketone was also shown to be present through its 3,4-dinitrophenylhydrazone. On the basis of the synthetic results and the reported presence of α-terpinene in chenopodium oil, α-terpinene is presumed to be the precursor for ascaridole in its biogenesis in the plant.

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CORRECTION

In the article entitled, "Synthesis of *dl*-Amphetamine Sulfate Labeled with C^{14}," by James W. Wilson, III [THIS JOURNAL, **39**, 687–688(1950)] in column 2, line 4, on page 687, "μc./mM" (microcuries per millimole) should appear as "mc./mM" (millicuries per millimole).

Further Studies on the Diffusion of Iodine and Sulfathiazole from Ointments*,†

By LAURENCE D. LOCKIE‡ and JOSEPH B. SPROWLS§

The influence of various factors upon the diffusion of iodine and sulfathiazole from ointment bases was studied, using a method previously described. Specifically studied were the influence of concentration variance, alteration of emulsifier, and the effect of the contact surface under the conditions of the experiment. It is concluded that for each of the drugs studied there is a concentration below which optimum diffusion conditions are not realized, and above which increased concentration of the drug does not lead to a significant increase in diffusion rate. Variation of the emulsifier in a standard base did not significantly alter the diffusion rate. The surface of contact was shown not to retard the diffusion of drug under the conditions of the experiment.

IN A PREVIOUS paper (1) it was suggested that the observed rate of diffusion of drugs from ointments into agar gels containing reagent may be expressed in parabolic curves of the form $y^2 = kx$, in which y represents the diffusion distance in millimeters and x the diffusion time in hours. It was also shown that the diffusion may be expressed in terms of linear equations in the form $z = kx$, in which z represents y^2 of the previous equation. It has been proposed that the value of k of the second equation be used as a "diffusion constant"[1] to indicate the ease with which a given drug is released from an ointment base.

It is the purpose of this paper to utilize the method of study previously described in determining the extent to which certain factors influence the rate of diffusion of drugs from ointment bases. Particular consideration will be given to the following factors: (*a*) the effect of drug concentration on diffusion rate; (*b*) effect of contact surface of ointment and agar gel (in the method of study) upon the rate of diffusion; and (*c*) effect of emulsifiers on the diffusion rate.

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‡ Associate Professor of Pharmacy, University of Buffalo.
§ Dean, Temple University School of Pharmacy.

[1] The value for k is related to, but is not to be confused with, D, the standard constant of diffusion which is calculated from Fick's law (2) in accordance with the equation:

$$dq = -DA\frac{c}{x}dt$$

where dq is the amount of material diffusing through a cross section of area A and of thickness δx in time dt and δc is the change in concentration over the distance δx. It should be noted, however, that the conclusion cited is entirely consistent with Fick's law as interpreted by Stefan as follows: the farthest distance to which the diffusing substance has penetrated must be proportional to the square root of the time that it has taken to get there.

PROCEDURE

The procedure used in the previous study is briefly reviewed here, since it is pertinent to the discussion which will follow. An agar gel (4%) is prepared containing suitable reagent (Ehrlich's solution 1% for sulfathiazole studies, and soluble starch 0.1% for iodine studies). The agar solution is poured into standard 6-in. test tubes, and when it is cool the ointment bases containing the desired strength of drug to be tested are superimposed upon the gel in a layer approximately 3 cm. deep. The depth of penetration of the drug into the agar gel after the lapse of definite time intervals is measured by the development of a color in the reagent gel. In our studies readings were taken at intervals up to one hundred and twenty hours; however, our results have indicated that seventy-two hours is sufficiently long to obtain reliable indications of diffusion rates. Formulas for all ointment bases may be found by consulting the previous publication of this series (1).

Effect of Drug Concentration on Diffusion Rates

In accordance with Fick's Law of Diffusion, the rate of release of drug from an ointment should be directly proportional to concentration; however, this generalization is approximately true only for dilute solutions in which the osmotic factor is the only significant factor which influences the movement of the particles. It seemed desirable to determine how the diffusion rate for the drugs under consideration might be influenced by concentration changes within the range of concentrations used therapeutically. Preliminary experiments performed with 5 and 20% sulfathiazole indicated no remarkable change in k with increase in concentration. The experimental data are revealed in Table I.

A consideration of these tables reveals that no measurable diffusion is obtained from sulfathiazole ointments prepared with grease bases, even when the strength is increased to 20%. Diffusion rates from oil-in-water bases are not significantly increased by an increase in percentage strength from 5 to 20. The average value of k for the 20% ointments is 12.4, compared with a value of 11.1 for the 5% ointments.

TABLE I.—DIFFUSION FROM 5 AND 20% SULFATHIAZOLE OINTMENTS

Base[a]	Diffusion Distance, Mm. Time, Hours: 1	3	6	24	48	72	96	120	Coefficient of x, k
A. 5%									
Petrolatum	0	0	0	0	0	0	0	0	0
Wool fat	0	0	0	0	0	0	0	0	0
Hydrophilic ointment	3	6	8	16	24	28	33	36	10.4
Vanishing cream "A"	4	6	7	17	25	28	34	38	11.8
Tragacanth paste	3	6	8	16	24	28	33	35	11.2
Carbowax base	5	8	10	18	25	36	41	45	
B. 20%									
Petrolatum	T[b]	T	T	T	0	0	0	0	0
Wool fat	2	3	5	7[a]	8[a]	15	Color gone		0
Hydrophilic ointment	4	7	9	17	25	30	34	36	12.4
Vanishing cream "A"	4	7	8	17	25	30	34	38	12.5
Tragacanth paste	3	7	8	17	25	30	33	35	12.4
Carbowax base	Not compatible in this concentration								

[a] Formulas for the ointment bases used in this work are printed in the first article in this series (1).
[b] T = Trace.

Because of the lack of significant variation in values of k with change in drug concentration, it was decided to carry on studies over a wider range of concentration using a standard base in order to reduce other variables to a minimum. Beeler's base[2] was selected because it is highly compatible with the drugs used, is easily prepared in a uniform condition, and is relatively stable. Sulfathiazole was used in concentrations of 0.1, 1.0, 2.5, 5.0, 10.0 and 20.0%. Iodine was used in concentrations of 0.1, 0.5, 1.0, 2.5, and 5%. Experimental results are presented in Table II.

TABLE II.—VARYING STRENGTHS OF SULFATHIAZOLE IN BEELER'S BASE

Strength, %	Distance, Mm. Time, Hours: 1	3	6	24	48	72	96	120	k
0.1	0	0	T	T	T	T	T	0	..
1.0	2	3	4	10	12	14	15	16	2.6
2.5	2	4	6	11	20	25	27	30	7.7
5.0	3	5	7	13	23	26	30	35	9.8
10.0	3	5	7	14	26	29	31	38	11.0
20.0	4	7	9	17	25	30	34	38	12.4
VARYING STRENGTHS OF IODINE IN BEELER'S BASE									
0.1	1	3	4	5	5	5	4	3	..
0.5	3	5	7	14	21	23	24	25	7.3
1.0	4	6	8	16	23	27	29	31	9.9
2.5	4	7	10	23	39	44	47	49	25.4
5.0	6	11	14	27	36	44	58	70	30.7

The change in coefficients of x are best visualized when presented in the form of a graph in which concentration of drug is plotted against values for k. Graphs of the results obtained are presented in Figs. 1 and 2.

A careful consideration of these data indicates that within the concentrations used for therapeutic purposes, no significant increase in rate of diffusion results from the use of the higher concentrations. With both drugs there is a marked increase in diffusion rate with increase in concentration in the lower concentrations; however, the values for k seem to level off at higher concentrations. These data strongly suggest the fact that for each drug there may be an optimum concentration beyond which increases in strength might not be justified by increased diffusibility, and below which the diffusibility decreases rapidly.

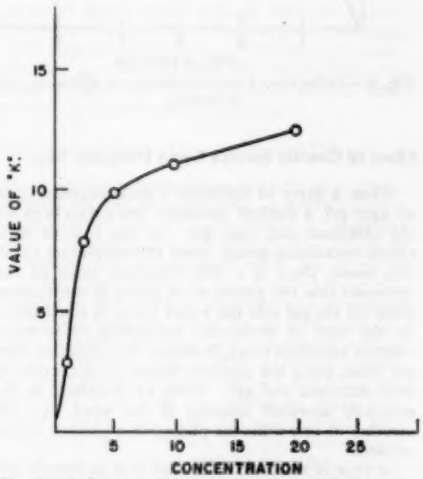

Fig. 1.—Influence of concentration on diffusion rate of sulfathiazole.

It is particularly significant that the maximum efficiency for both drugs is reached at concentrations within the range which have been found to be most efficacious when tested clinically. Thus, sulfathiazole seems to reach a maximum of efficiency (in so far as diffusion is concerned) at a concentration range of 10 to 20%.

For iodine, the maximum efficiency is reached at approximately 5%, a concentration only slightly higher than the concentration of Iodine Ointment, N. F. Since Iodine Ointment, N. F., does not contain an aqueous base, the latter coincidence may have no significance.

[2] The formula for Beeler's base is as follows (3):

Cetyl alcohol	15.0 Gm.
White wax	1.0 Gm.
Propylene glycol	10.0 Gm.
Sodium lauryl sulfate	2.0 Gm.
Water	72.0 Gm.

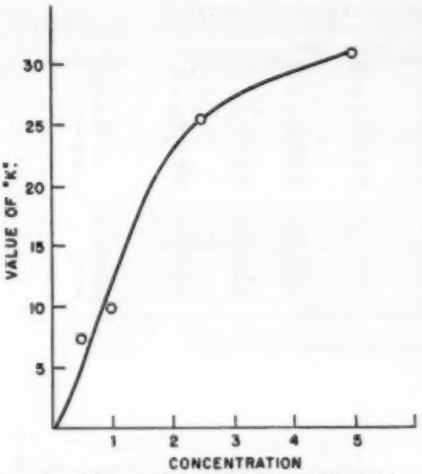

Fig. 2.—Influence of concentration on diffusion rate of iodine

Effect of Contact Surface Upon Diffusion Rate

When a layer of ointment is superimposed upon an agar gel, a distinct boundary line exists between the ointment and agar gel. In the case of ointments containing grease bases or water-in-oil emulsion bases, there is a true interface, since on the ointment side the grease or oil phase is continuous, while on the gel side the water phase is continuous. In the case of ointments containing oil-in-water bases or mucilage bases, however, this situation does not exist, since the aqueous phase is continuous in both ointment and gel. Thus, an interface, in the generally accepted meaning of the word, i.e., the meeting of two different phases of a system, is not present.

In view of what has been said, it is extremely unlikely that the contact surface between ointment and agar gel would exert any influence upon the diffusion of drugs from oil-in-water base or mucilage base ointments in the method of testing used. Nevertheless, it was decided to conduct an experiment which would determine the validity of the assumption which has just been expressed.

MODIFIED PROCEDURE

The procedure previously discussed was modified, as will be described. Tubes of reagent agar gel were prepared as before and upon these were superimposed varying depths of ointment base containing no drug. Over the layer of ointment base was superimposed a thin layer of reagent gel. Topping the whole was a layer of 5% aqueous solution of iodine containing potassium iodide. Thus, there was set up a system in which drug must diffuse through a thin layer of reagent agar gel, then through a depth of plain ointment base, then into a column of reagent agar gel. Iodine was used because of its fast rate of diffusion which made it possible to shorten the time of experimentation. The upper layer of reagent gel was provided to prevent the aqueous solution from mixing with the ointment base, to make possible an accurate observation of the time of entry of drug into the ointment base, and to provide for a second surface of contact so that any effect would be doubled and thereby exaggerated. A diagram of the arrangement is shown in Fig. 3.

The time at which the iodine reached the ointment layer was noted (point 1 in Fig. 3), as was the time when it emerged from the ointment base (point 2 in Fig. 3). After the iodine entered the lower agar gel, readings were taken at twenty-four, forty-eight, and ninety-six hours in order to follow the progress of drug after emergence from the ointment base. The data obtained from a series of depths of sodium lauryl sulfate base by this method are shown in Table III. These data were then interpreted in terms of the equation $z = kx$, in which z equals the square of the distance in millimeters and in which x equals the time of diffusion. Plotting values of z as ordinates and values of x as abscissas, as is done in Fig. 4, makes it possible to determine the value of k, since it is equivalent to the slope of the line.

TABLE III.—EFFECT OF CONTACT SURFACE ON DIFFUSION

(5% Iodine Solution Sodium Lauryl Sulfate Base)

	Sample			
	A	B	C	D
Upper agar layer (mm.)	5	5	5	5
Time through upper agar layer (hr.)	0.5[a]	0.5	0.5	0.5
Ointment layer (mm.)	3	5	8	9
Time through ointment layer (hr.)	3	6	9	13
Distance in lower agar gel (mm.)				
24 hr.	15	10	8	5
48 hr.	30	22	19	14
96 hr.	42	38	33	20

[a] = two minutes.

Discussion of Results.—Two contact surfaces were provided by the arrangement shown in Fig. 3. The following results would be possible, due to the contact surface: (*a*) to stop the diffusion completely; (*b*) to retard the diffusion; (*c*) to have no effect on the diffusion; and (*d*) to accelerate the diffusion. The first result was known not to take place, since diffusion had been obtained in all experiments. The fourth result seems unlikely. The other two possibilities require further discussion.

When the diffusion of drugs from ointment bases is expressed in the form of straight line graphs obtained by graphing observed results in terms of the equation $z = kx$, differences in diffusion rates are indicated by differences in the slopes of the graphs. Now the same drug, under the same conditions, would always have the same diffusion rate; hence, the same graph and the same slope, even though the total distance traveled through the ointment base were different, unless the contact surface exerts some effect. This effect would be to add a constant amount of time to the time necessary for the iodine to traverse the ointment layer if the contact surface retarded diffusion, or to subtract a constant amount of time if the contact surface accelerated diffusion. If this constant amount of time were added to (or subtracted from) the time necessary for

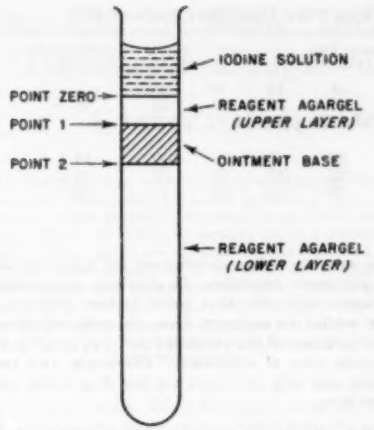

Fig. 3.—Diagram of system used in studying effect of contact surface on diffusion.

the iodine to traverse successively thicker ointment layers, the slope of the graph for each layer of ointment should be successively smaller (or greater).

The slopes of the lines indicating the rate of passage through ointment base were now calculated using the customary equation for the slope of a line between two points (corresponding to point 1 and point 2 in Fig. 3). The equation used is as follows:

$$\frac{z_2 - z_1}{x_2 - x_1} = k^2$$

where z_2 = square of distance in mm. traveled in reaching point 2, Fig. 2; z_1 = square of distance in mm. traveled in reaching point 1; x_2 = time in hours required to traverse to point 2; and x_1 = time in hours required to reach point 1. The slopes obtained are presented in Table IV.

A graphical representation of the data from Table III, and from which the slopes may be read, is presented in Fig. 4.

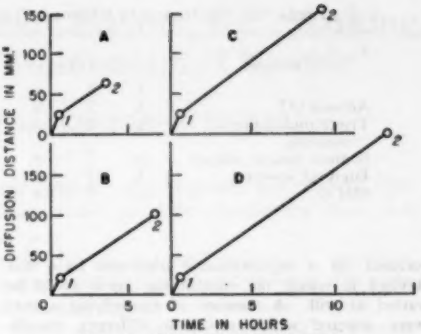

Fig. 4.—Slopes from Table III.

These slopes do not exhibit the successive increase or decrease which would indicate that the contact surface is exerting an effect upon the rate of diffusion of drug through ointment base; therefore, it seems reasonable to conclude that any effect which the contact surface may have upon the rate of diffusion must be insignificant in character.

Effect of Emulsifying Agent on Rate of Diffusion

Many recent investigations have focused attention upon the use of emulsifying or wetting agents (particularly of the synthetic type) in ointment bases. The improvement of the physical nature of an ointment base which results when the oil phase is properly distributed in the form of very fine globules and the increased stability of the resulting emulsion are sufficient reason for justifying interest in the emulsified ointment bases containing wetting agents. However, there is little information available concerning any influence which different wetting agents may have upon the release of drugs from ointment bases. In most instances where such studies have been reported, the emulsifying agent has not been the only variable in the different bases used. For example, any differences between Beeler's base, which contains sodium lauryl sulfate, and Elvin's base, which contains glyceryl monostearate, cannot be attributed solely to the change in emulsifying agent.

In order to determine the influence which alteration of emulsifying agent might have upon the rate of diffusion of drug from ointment base, a basic

TABLE IV

Sample	Depth of Ointment Base, Mm.	Slope Between Points 1 and 2[a]
A	3	13.0
B	5	12.5
C	8	13.2
D	9	13.1

[a] A simple calculation reveals the fact that an increase of 15 minutes (0.25 hr.) in the diffusion time for sample A would decrease the slope from 13.0 to 12.0. A similar decrease for sample B would increase the slope from 12.5 to 13.05. Thus, the variations in slope which do exist could in no instance reflect a delay of more than 15 minutes due to surface effects. In view of the close similarity of the observed slopes, we believe that the slight variations may be accepted as experimental variations.

[5] This equation may also be derived upon a theoretical basis in the following fashion: in accordance with Fick's Law of Diffusion it may be assumed that the farthest distance to which a diffusing substance has penetrated is proportional to the square root of the time taken to get there. In other words,

$$y \text{ (distance)} = k\sqrt{x} \qquad (x = \text{time})$$

or

$$y^2 \alpha x$$

In our experiment the iodine diffuses through two different media (agar and ointment gel) and its passage is governed by two constants, one of which influences the other. This may be represented mathematically (using the letters as just indicated) as follows:

$(y_1)^2 = k_1 x_1$ (for the agar gel)
$(y_2)^2 = (y_1)^2 + k_2(x_2 - x_1)$ (for agar gel plus ointment base)

Solving

$$\frac{(y_2)^2 - (y_1)^2 = k_2(x_2 - x_1)}{x_2 - x_1} = k_2$$

Substituting z_2 and z_1 for $(y_2)^2$ and $(y_1)^2$

$$\frac{z_2 - z_1}{x_2 - x_1} = k_2 \text{ (the slope between point 1 and point 2).}$$

TABLE V.—1% IODINE IN UNIFORM FORMULA BASE WITH DIFFERENT EMULSIFIERS

Emulsifier	Distance, Mm. — Time, Hours: 1	3	6	24	48	72	96	120	Coefficient of x, k
Aerosol OT	4	7	9	19	27	37	40	43	16.8
Triethanolamine stearate	4	6	Color disappeared						
Sodium lauryl sulfate	4	7	9	18	26	33	36	40	14.1
Diglycol stearate	4	7	9	18	25	32	35	37	13.4
SHCO	4	7	9	18	26	34	38	41	15.0

formula for a representative ointment base was devised in which the emulsifying agent could be varied at will. A number of emulsifying agents were selected which represent different classifications of emulsifiers and these were added to the standard ointment base. The formula for the standard ointment base used is as follows:

White wax	5 Gm.
Cetyl alcohol	5 Gm.
Heavy liquid petrolatum	15 Gm.
Emulsifier	3 Gm.
Water	72 Gm.

The fats were melted together and heated to about 70°. The emulsifier was dissolved or mixed with the water and heated to the same temperature. The two solutions were mixed, and stirred until cool. If necessary, a mechanical mixer was used to assist in obtaining a satisfactory emulsion. Obviously the bases were not uniform in appearance, since the nature of the emulsifier used often influences the nature of an emulsion. Iodine (1%) was added to all bases. The emulsifiers used were as follows:

Sodium lauryl sulfate (anionic)
Diglycol stearate (nonanionic)
Aerosol OT (anionic)
Sulfated hydrogenated castor oil
Triethanolamine stearate (cationic)

Values for k were determined for these ointments in the same manner as before and are reported in Table V.

Discussion of Results.—The most striking result of this study is the similarity in values of k for the bases containing different emulsifiers. This is particularly significant when compared to previous results in which ointment bases were compared which contained other variable ingredients in addition to the wetting agent. However, the values for k for these bases are slightly higher than were the values determined for bases containing the same emulsifiers in previous experiments. It seems apparent that other factors which may vary within an ointment base are more significant than variation of the emulsifier *per se* in so far as the diffusion rate is concerned. Obviously this conclusion can only be drawn for the drug under consideration.

CONCLUSIONS

1. The data obtained in these experiments indicate that for sulfathiazole and iodine there is an optimum concentration beyond which increases in strength do not produce increased diffusibility, and below which the diffusibility decreases rapidly under the conditions of the experiment.

2. For sulfathiazole ointments, the optimum advantage was obtained at concentrations in the neighborhood of 5 per cent. For iodine, the most practical concentration was found to be around 2.5 per cent.

3. It has been demonstrated that, for oil-in-water emulsion bases, the surface of contact with agar gel has no influence upon the rate of diffusion of drug from ointment base under the conditions of the test.

4. Change in emulsifying agents in a standard ointment base appears to have little influence upon the rate of diffusion of iodine.

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The Use of Some Imitation Flavors for Masking Distasteful Drugs. I. Ammonium Chloride*

By BETTY LOUISE LANKFORD† and CHARLES H. BECKER‡

An investigation was made to determine the relative efficiencies of some imitation flavor concentrates actually prepared from the basic ingredients as masking agents on the initial and obnoxious tastes of ammonium chloride. These were compared to some of the official flavored syrups recognized as good masking agents for saline drugs. Results show that some imitation flavored syrups were as good as the official syrups used and in some instances superior.

ALTHOUGH some work has been done in the past on the evaluation of imitation flavors purchased from flavor manufacturers for disguising drugs, little or no research has been carried out to evaluate the disguising powers of imitation flavors such as grape, raspberry, root beer, etc., actually prepared from known materials in the laboratory. It was the purpose of this investigation to determine the efficacy as disguising agents for a salty drug, ammonium chloride, of some imitation flavors actually produced from essential oils, aromatics, etc., that are generally employed in making imitation flavors.

Many different flavored syrups are official for masking obnoxious-tasting drugs; however, the majority are unsatisfactory. It was felt that there is a need for flavored syrups prepared from imitation concentrates since these would be more consistent and standard, less costly, and more easily obtainable. These would provide a greater variety of flavors than the official ones from which the physician could choose according to the desires of the patient, and which also would be more desirable for children. The fact that imitation flavors are becoming increasingly popular is illustrated by their appearance in manufacturers' products and by their being specified on prescriptions by physicians (1).

HISTORY

Purdum (2), working with official flavored syrups, found the taste of ammonium chloride was disguised most effectively by syrups of glycyrrhiza, raspberry, orange flower, citric acid, and tolu balsam. The disguising potentials of these vehicles did not differ significantly from each other.

Wright (3) reported syrups of cinnamon, orange, and sarsaparilla effective in that order as masking agents for ammonium chloride.

Libitr and co-workers (4) found by organoleptic tests that cranberry syrup prepared from the fresh fruit was particularly effective as a masking agent for salty drugs.

Fantus, *et al.* (5) recommended the glycyrrhiza preparations as vehicles for salty drugs.

Other vehicles reported as efficient masking agents for salty drugs are syrups of fresh pineapple (6) and acacia (7).

EXPERIMENTAL

Materials and Formulas of Imitation Concentrates.—The majority of the chemicals employed in the preparation of the imitation flavor concentrates were those generally employed by the flavor manufacturers. They were of food grade. All others were of U. S. P. or N. F. quality. The manufacturers of the chemicals that were not official are indicated in the formulas by the following abbreviations in parentheses following the chemicals: Florasynth Laboratories, Inc. (Fl), Fritzsche Brothers, Inc. (Fr), Synfleur Laboratories, Inc. (S), and S. B. Penick and Co. (P).

When extremely small quantities of the chemicals were called for, 10% or 1% tinctures were made, and aliquot portions taken to insure accurate measurements. The concentrates were made by simple solution, as all chemicals were soluble in the solvents used.

The formulas for the imitation flavor concentrates used appear below.

IMITATION GRAPE CONCENTRATE

Methyl Anthranilate (Fr)	4.55	cc
Dimethyl Anthranilate (Fr)	0.36	cc.
Rose Oil (Rosalik) (Fr)	0.036	cc.
Jasmine-W (S)	0.072	cc.
Propylene Glycol	4.1	cc.
Ethyl Acetate, a sufficient quantity, to make	100	cc.

IMITATION ROOT BEER CONCENTRATE

Methyl Salicylate	6.25	cc.
Sassafras Oil	2.25	cc.
Cinnamon Oil	0.25	cc.
Anise Oil	0.37	cc.
Clove Oil	0.80	cc.
Alcohol	2.25	cc.
Propylene Glycol, a sufficient quantity, to make	100	cc.

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† This paper is based on part of a thesis presented to the Graduate Council of the University of Florida by Betty Louise Lankford in partial fulfillment of the requirements for the degree of Master of Science in Pharmacy.

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IMITATION BUTTERSCOTCH CONCENTRATE

Vanillin	1.5	Gm.
Foenugreek Extract (P)	1.35	Gm.
Butyric Acid (P)	0.25	cc.
Caproic Acid (S)	0.25	cc.
Diacetyl (S)	1	cc.
Ethyl Myristate (Fl)	1	cc.
Lovage Oil (P)	0.0015	cc.
Orange Oil	0.5	cc.
Tar Oil, Rectified	0.006	cc.
Ginger Oil (Fr)	0.25	cc.
Propylene Glycol, a sufficient quantity, to make	100	cc.

IMITATION MAPLE CONCENTRATE

Foenugreek Extract (P)	25	Gm.
Lovage Oil (P)	0.025	cc.
Imitation Butterscotch Concentrate (above formula)	6.66	cc.
Propylene Glycol	37.5	cc.
Distilled Water, a sufficient quantity, to make	100	cc.

IMITATION RASPBERRY CONCENTRATE

Vanillin	0.18	Gm.
Indol (Fr)	0.004	Gm.
Aldehyde C_{18} (Fr)	0.24	cc.
Diacetyl (S)	0.06	cc.
Phenyl Ethyl Alcohol (Fr)	0.24	cc.
Aldehyde C_{14} (S)	0.015	cc.
Aldehyde C_{18} (Fr)	0.015	cc.
Aldehyde C_{20} (Fr)	0.40	cc.
Orange Flower Oil	0.006	cc.
Ethyl Butyrate (Fr)	0.12	cc.
Benzyl Acetate (S)	0.075	cc.
Novoviol, *Alpha* (Fr)	0.4	cc.
Novoviol, *Beta* (Fr)	0.2	cc.
Lemon Oil	0.06	cc.
Propylene Glycol, a sufficient quantity, to make	100	cc.

IMITATION CREAM SODA CONCENTRATE

Vanillin	12	Gm.
Coumarin	1.2	Gm.
Heliotropin (Fr)	1.2	Gm.
Orange Flower Oil	0.25	cc.
Diacetyl (S)	1	cc.
Linaloöl, from Bois de Rose (Fr)	0.5	cc.
Propylene Glycol, a sufficient quantity, to make	100	cc.

IMITATION WILD CHERRY CONCENTRATE

Vanillin	0.55	Gm.
Coumarin	0.17	Gm.
Amyl Acetate (S)	0.13	cc.
Anise Oil	0.1	cc.
Glacial Acetic Acid	0.23	cc.
Benzaldehyde	8.3	cc.
Propylene Glycol, a sufficient quantity, to make	100	cc.

IMITATION COCONUT CONCENTRATE

Aldehyde C_{18} (Fr)	10 cc.
Propylene Glycol, a sufficient quantity, to make	100 cc.

Preparation of Syrups.—The imitation flavored syrups were prepared by adding 1.67 cc. of the imitation concentrate to enough simple syrup to make 1,000 cc. The acidified imitation flavored syrups were prepared by adding 1.67 cc. of the imitation concentrate and 4.00 Gm. of citric acid to enough simple syrup to make 1,000 cc.

Inasmuch as Purdum (2) found that syrups of glycyrrhiza, raspberry, cacao, and aromatic eriodictyon were the most effective masking agents in his investigation, it was decided to use them here as controls. Likewise, cherry syrup was used as a control. The aromatic eriodictyon, raspberry, and cherry syrups were purchased from a pharmaceutical house, whereas the cacao and glycyrrhiza syrups were prepared in the laboratory.

Concentrations of Solutions of Ammonium Chloride Used.—Ten concentrations of the drug were used. The normalities ranged from 0.0044 to 0.17, increasing proportionally so that the ratio of 1:1.5 existed between adjacent solutions. The concentrations were numbered from 1 to 10, respectively, in ascending order of normalities. The numbers of the solutions with the corresponding normalities and the amount of ammonium chloride contained in the 4-cc. portions of each strength which served as samples for the taste tests are listed in Table I.

TABLE I.—TEN CONCENTRATIONS OF AMMONIUM CHLORIDE USED

Solution No.	Normality	Concentration of Drug in 4-Cc. Taste Test Sample, Gm.
1	0.0044	0.0009
2	0.0067	0.0014
3	0.010	0.0021
4	0.015	0.0032
5	0.022	0.0048
6	0.033	0.0072
7	0.050	0.011
8	0.075	0.016
9	0.11	0.024
10	0.17	0.036

Preparation of Taste Test Solutions and Controls.—The vehicular solutions were prepared as follows: a quantity of the ammonium chloride solution, containing a known amount of the salt, was mixed with 1 fl. oz. of the flavored syrup or simple syrup and then enough distilled water was added to make 6 fl. oz. In other words, in preparing taste solution no. 1 of the vehicular solution, a known quantity of ammonium chloride solution containing 0.041 Gm. of the salt was added to 1 fl. oz. of simple syrup or flavored syrup and then enough distilled water was added to make 6 fl. oz. Four cubic centimeters of this solution, which was the taste test sample, would contain 0.0009 Gm. of ammonium chloride.

The aqueous solutions of ammonium chloride were prepared in the same manner as described above, except that only distilled water was used as the vehicle for the ammonium chloride to make up to 6 fl. oz.

The controls were prepared by mixing 1 fl. oz. of the flavored syrup or simple syrup and 5 fl. oz. of distilled water. The ammonium chloride was left out of the control solutions.

In order to prevent possible psychological effects, no attempt was made to color the imitation flavored syrups to resemble the natural products. Thus, all solutions prepared with the imitation flavored concentrates were practically colorless. It was felt that the effectiveness of the imitation flavors would be more correctly evaluated in this manner.

The Method of Evaluation of the Vehicles.—Purdum's method (2), with a few modifications, was used in conducting the taste tests for the evaluation of the vehicles. The vehicular solutions used by Purdum were 10%, by volume, of the vehicle in water. In this investigation the vehicular solutions were 16.66% of the flavored syrups in water.

Whereas a minimum of 30 adult persons served as subjects for Purdum, the same group of people being maintained throughout a series of tests, a minimum of 15 adult subjects were used here, and no effort was made to maintain the same group throughout the entire series of tests.

In addition to finding out the mean disguising potentials of the vehicles, the mean obnoxious potentials were also determined.

Subjects for the taste tests consisted of adults, male and female, who were in good general health and who were not affected by anodmia, rhinitis, allergies such as hayfever, or other conditions which could interfere with the senses of taste and smell. Subjects who were fatigued due to strenuous exercise were also not used.

The taste tests were conducted in a well-ventilated room free from foreign odors. The temperature was maintained at average room temperature.

All solutions for the taste tests and the distilled water for rinsing the mouth were of room temperature. The aqueous solutions and vehicular solutions of the ammonium chloride and controls, in 4-cc. taste test portions of each strength of ammonium chloride, were placed in numbered 50-cc. beakers set up in numerical order. These numbers corresponded to the concentrations of the ten concentrations of ammonium chloride.

The taste threshold and obnoxious threshold of each subject was determined in distilled water solutions of ammonium chloride on each working day. The subject first rinsed his mouth with a control of a 4-cc. portion of distilled water. This acquainted him with the tastelessness of distilled water and also with the temperature of all solutions that were to be tasted. The subject was then requested to taste each sample of the aqueous solutions in ascending numerical order, as indicated by the numbers of the beakers, until the taste threshold and the obnoxious threshold were reached. The taste threshold was defined to the subject as the number of the concentration at which the distilled water ceased to be tasteless, as compared to the control. The obnoxious threshold was defined as the number of the concentration at which the subject found it undesirable for oral use.

The entire amount of the 4-cc. taste test sample was taken into the mouth and was thoroughly rinsed over all regions of the mouth so that all taste buds would be touched. The sample was then ejected from the mouth and the mouth thoroughly rinsed with distilled water. The subject was cautioned to rinse from the mouth all aftertastes of the drug from the preceding sample before continuing to the next taste test. When the subject was unable to decide if a threshold was reached, he was given another sample of the same concentration to retaste. If he were still uncertain, he tasted the next higher concentration and recorded that as the threshold.

The thresholds for the vehicular solutions were determined in a similar manner. Four-cubic centimeter taste test portions of each strength of ammonium chloride and the controls were placed in numbered 50-cc. beakers set up in numerical order. Since all vehicular solutions of the same number contained the same concentration of drug in solution, and this number corresponded with the number of the aqueous solution of the same concentration, the vehicles were compared directly with the aqueous solutions.

TABLE II.—THE EFFECTIVENESS OF SOME IMITATION FLAVORED AND OFFICIAL SYRUPS ON THE DISGUISING POTENTIALS FOR AMMONIUM CHLORIDE

Syrup	No. of Subjects	Mean Threshold in Water ± Standard Error $m_2 \pm \epsilon_2$	Mean Threshold in Syrup ± Standard Error $m_1 \pm \epsilon_1$	Mean Disguising Potential ± Standard Error $D \pm \epsilon$	Significance of Difference
Raspberry syrup, N. F.	17	3.41 ± 0.33	6.65 ± 0.45	3.24 ± 0.40	5.83
Imitation raspberry syrup, acid added	16	3.38 ± 0.36	6.50 ± 0.43	3.12 ± 0.56	5.56
Imitation wild cherry syrup, acid added	15	3.20 ± 0.29	6.07 ± 0.41	2.87 ± 0.53	5.52
Imitation grape syrup, acid added	16	3.38 ± 0.36	6.13 ± 0.43	2.75 ± 0.52	4.90
Cacao syrup, N. F.	15	3.67 ± 0.37	6.20 ± 0.59	2.53 ± 0.57	3.62
Cherry syrup, N. F.	17	3.65 ± 0.38	6.00 ± 0.52	2.35 ± 0.40	3.64
Imitation maple syrup	15	3.67 ± 0.40	6.00 ± 0.36	2.33 ± 0.27	4.38
Glycyrrhiza syrup, U. S. P.	15	3.53 ± 0.39	5.73 ± 0.56	2.20 ± 0.42	3.26
Imitation butterscotch syrup	15	3.66 ± 0.36	5.73 ± 0.42	2.07 ± 0.44	3.74
Aromatic eriodictyon syrup, N. F.	17	3.82 ± 0.38	5.88 ± 0.49	2.06 ± 0.42	3.34
Imitation coconut syrup	17	3.83 ± 0.37	5.88 ± 0.34	2.05 ± 0.18	4.17
Imitation cream soda syrup	15	3.80 ± 0.41	5.53 ± 0.44	1.73 ± 0.37	2.82
Imitation wild cherry syrup	18	3.78 ± 0.34	5.50 ± 0.39	1.72 ± 0.27	3.30
Imitation grape syrup	16	3.50 ± 0.41	5.19 ± 0.53	1.69 ± 0.40	2.57
Imitation root beer syrup	16	3.88 ± 0.38	5.31 ± 0.31	1.43 ± 0.28	2.88
Imitation raspberry syrup	16	3.88 ± 0.38	5.19 ± 0.43	1.31 ± 0.34	2.28
Simple syrup, U. S. P.	16	3.88 ± 0.35	4.94 ± 0.36	1.06 ± 0.29	2.08

TABLE III.—THE SIGNIFICANCE OF DIFFERENCES BETWEEN THE MEAN DISGUISING POTENTIALS FOR AMMONIUM CHLORIDE WITH IMITATION FLAVORED AND OFFICIAL SYRUPS LISTED IN TABLE II

Raspberry syrup, N. F.	Imitation raspberry syrup, acid added	Imitation wild cherry syrup, acid added	Imitation grape syrup, acid added	Cacao syrup, N. F.	Cherry syrup, N. F.	Imitation maple syrup	Glycyrrhiza syrup, U. S. P.	Imitation butterscotch syrup	Aromatic eriodictyon syrup, N. F.	Imitation coconut syrup	Imitation cream soda syrup	Imitation wild cherry syrup	Imitation grape syrup	Imitation root beer syrup	Imitation raspberry syrup	Syrup, U. S. P.
0.17																
0.56	0.32															
0.74	0.49	0.16														
1.03	0.75	0.44														
1.56	1.01	0.79														
1.80	1.27	0.92														
1.82	1.33	1.17														
1.98	1.48	1.16														
2.04	1.49	1.18														
2.70	1.85	1.46														
2.74	2.04	1.75														
3.17	2.26	1.95														
2.73	2.04	1.79														
3.69	2.72	2.39														
3.65	2.75	2.48														
4.36	3.30	2.97														
			0.29	0.26	0.04	0.27	0.22	0.02	0.02	0.78	0.02	0.06	0.83	0.27	0.54	
			0.64	0.32	0.26	0.51	0.24	0.04	0.58	1.03	0.07	0.74	0.72	0.90		
			0.76	0.47	0.48	0.54	0.34	0.60	0.68	0.82	0.64	0.93	1.26			
			0.83	0.65	0.50	0.87	0.84	0.69	0.64	0.64	1.23	0.83				
			1.00	0.66	0.68	1.30	0.97	0.64	1.23	1.88	1.35	1.65				
			1.04	1.37	1.13	1.65	0.90	1.23	1.36	1.90	1.91	2.32				
			1.27	1.18	1.31	1.33	1.54	1.35	1.92	2.82						
			1.59	1.31	1.16	2.31	1.65	1.91								
			1.78	1.20	1.67	2.32	2.23									
			1.61	1.73	1.96	3.18										
			2.27	1.85	2.58											
			2.33	2.30												
			2.82													

Threshold values of several vehicles could be determined on the same working day for a subject. The person was cautioned to discontinue the tests if a desensitizing effect on the taste buds began to appear, or if the aftertaste of the ammonium chloride or the aftertaste of the flavored vehicle became too persistent to remove by rinsing.

The disguising powers of the vehicles were determined by taking the differences between the mean thresholds in the aqueous solutions and vehicular solutions. According to Purdum (2), the mean differences for subjects should remain approximately the same even though the subjects developed a keener sense of taste or acuity for the drug. If a subject's taste threshold appeared at concentration No. 3 in the aqueous solution, and at concentration No. 7 in a vehicular solution, the disguising potential of that vehicle for the subject would be 4. If, upon repeating this test on the same vehicle after several weeks and the values of 2 and 6 were found for the respective thresholds, the result would still be the same, since in either case the disguising potential is 4.

This method established the relative differences between the threshold values in the aqueous and each vehicular solution. The mean differences were expressed numerically, since the solutions of ammonium chloride were numbered in such a manner that all solutions of the same number of each concentration of ammonium chloride, aqueous and vehicular, contained the same amount of the salt, this concentration being proportional in the same ratio for any set of consecutively numbered solutions. Therefore, the concentrations of the solutions were of no significance other than to furnish data from which the differences could be determined.

The formula $\sqrt{\Sigma d^2/n(n-1)}$ was used to determine the standard errors for each set of determinations. The deviation of each individual from the mean, whether plus or minus, was represented by d. The sum of all these deviations squared is represented by d^2. The number of subjects in each set of determinations was represented by n.

The formula $(m_1 - m_2)/\sqrt{\epsilon_1^2 + \epsilon_2^2}$ was applied to determine whether the mean thresholds of the aqueous and vehicular solutions differed significantly. A value of 1.96 or greater indicates a significant difference. The means are represented by m_1 and m_2 for the vehicular and aqueous solutions, respectively. If a value of 1.96 or greater was obtained, this indicated that the means differed significantly, and not through inherent variations in the subjects.

TABLE IV.—THE EFFECTIVENESS OF SOME IMITATION FLAVORED AND OFFICIAL SYRUPS ON THE OBNOXIOUS POTENTIALS FOR AMMONIUM CHLORIDE

Syrup	No. of Subjects	Mean Threshold in Water ± Standard Error $m_2 \pm \sigma_2$	Mean Threshold in Syrup ± Standard Error $m_1 \pm \sigma_1$	Mean Obnoxious Potential ± Standard Error $D \pm \delta$	Significance of Difference
Imitation cream soda syrup	15	7.13 ± 0.44	9.20 ± 0.31	2.07 ± 0.49	3.99
Imitation maple syrup	15	7.60 ± 0.46	9.67 ± 0.16	2.07 ± 0.54	4.41
Glycyrrhiza syrup, U. S. P.	15	7.13 ± 0.43	9.20 ± 0.32	2.07 ± 0.56	3.83
Raspberry syrup, N. F.	17	7.65 ± 0.50	9.65 ± 0.12	2.00 ± 0.53	3.92
Cacao syrup, N. F.	15	7.47 ± 0.17	9.20 ± 0.22	1.73 ± 0.14	2.02
Cherry syrup, N. F.	17	7.82 ± 0.50	9.47 ± 0.18	1.65 ± 0.56	3.12
Imitation butterscotch syrup	15	7.87 ± 0.49	9.40 ± 0.16	1.53 ± 0.46	2.95
Imitation root beer syrup	16	8.25 ± 0.33	9.62 ± 0.13	1.37 ± 0.31	3.92
Imitation wild cherry syrup	18	7.72 ± 0.31	8.94 ± 0.34	1.22 ± 0.32	2.66
Imitation grape syrup, acid added	16	8.25 ± 0.37	9.44 ± 0.18	1.19 ± 0.37	2.90
Imitation raspberry syrup, acid added	16	8.25 ± 0.37	9.44 ± 0.18	1.19 ± 0.43	2.90
Imitation wild cherry syrup, acid added	15	8.40 ± 0.39	9.53 ± 0.17	1.13 ± 0.41	2.70
Imitation coconut syrup	17	8.12 ± 0.40	9.24 ± 0.24	1.12 ± 0.38	2.39
Imitation grape syrup	16	7.25 ± 0.45	8.25 ± 0.42	1.00 ± 0.38	1.62
Imitation raspberry syrup	16	8.13 ± 0.37	9.07 ± 0.34	0.94 ± 0.39	1.91
Aromatic eriodictyon syrup, N. F.	17	7.88 ± 0.44	8.82 ± 0.31	0.94 ± 0.48	1.77
Syrup, U. S. P.	16	8.13 ± 0.37	8.81 ± 0.38	0.68 ± 0.42	1.64

The latter formula was again employed to determine which vehicles varied significantly as disguising agents for ammonium chloride.

Results of Table II show that the significance of difference (last column) between the mean threshold for a vehicular solution and an aqueous solution of ammonium chloride is 1.96 or greater for all vehicles used in this series. A value of 1.96 or greater indicates that the difference is significant, and not due to inherent variations in the subjects. It is also seen that the imitation fruit-flavored syrups—wild cherry, grape, and raspberry—were poor disguising vehicles. The addition of citric acid to these syrups greatly increased their mean disguising potentials. In fact, with the exception of raspberry syrup, N. F., they had the highest mean disguising potentials. Of the official flavored syrups used, raspberry was the best. Cacao, cherry, aromatic eriodictyon, and glycyrrhiza syrups, previously recognized as excellent disguising vehicles, were not found as good as the imitation fruit-flavored syrup with acid added.

In Table III the vehicles are arranged in order of decreasing mean disguising potentials. Values of 1.96 or greater indicate a significant difference. Results show that all syrups from raspberry syrup, N. F. to, and including, glycyrrhiza syrup, U. S. P., were considered the most effective masking agents of all vehicles used; however, none in the group was considered better than the other. All vehicles falling below glycyrrhiza syrup, U. S. P., were inferior to those above.

Results of Table IV show that imitation grape, imitation raspberry, aromatic eriodicyton, and simple syrups were not significantly better than water for masking the obnoxious taste of ammonium chloride. This is indicated by their having values of less than 1.96. All the rest of the syrups were significantly better than water. The addition of citric acid to the imitation fruit-flavored syrup did not enhance these vehicles appreciably as far as masking the obnoxious taste of ammonium chloride. It is also seen from the results that all of the syrups did not have too high a safety factor between the masking of the initial taste and the obnoxious taste of ammonium chloride.

Results of Table V show that imitation cream soda syrup, imitation maple syrup, glycyrrhiza syrup, U. S. P., raspberry syrup, and cacao syrup, N. F., were the only vehicles found to be significantly better than syrup, U. S. P., for masking the obnoxious taste of ammonium chloride. Not any in this group could be considered better than the other. All syrups below cacao syrup, N. F., were definitely inferior to the above. The addition of acid to the imitation fruit-flavored syrups did not improve them for masking the obnoxious taste of ammonium chloride.

DISCUSSION

Results show that the imitation flavored syrups used in this investigation are as effective as and, in some instances, more effective than the official syrups for masking the taste of ammonium chloride. It appears that flavors effective for masking the distastefulness of a drug of one type, such as a salty drug, should also be effective on another drug of the same type. This presents possibilities for further research.

It was also found that flavors which would mask the initial salty taste of ammonium chloride would not always mask the obnoxious taste of the drug. For this reason, the mean disguising potentials of flavors cannot always be used as a basis for selecting a suitable vehicle for a drug.

The addition of acid to the imitation fruit flavors, as a whole, increased the mean disguising and obnoxious potentials of ammonium chloride. The reason for this is probably due to the fact that the "flatness" of a plain solution of these flavors is changed to a tartness by the addition of acid, and this tartness temporarily inhibits the taste buds of the mouth.

The concentrations of the flavors, sugar, and acid in the imitation flavors were kept constant throughout the investigation. Further research as to the effects of increasing these concentrations should yield promising results.

TABLE V.—THE SIGNIFICANCE OF DIFFERENCES BETWEEN THE MEAN OBNOXIOUS POTENTIALS FOR AMMONIUM CHLORIDE WITH IMITATION FLAVORED AND OFFICIAL SYRUPS LISTED IN TABLE IV

	Imitation cream soda syrup	Imitation maple syrup	Glycyrrhiza syrup, U. S. P.	Raspberry syrup, N. F.	Cacao syrup, N. F.	Cherry syrup, N.F.	Imitation butterscotch syrup	Imitation root beer syrup	Imitation wild cherry syrup	Imitation grape syrup, acid added	Imitation raspberry syrup, acid added	Imitation wild cherry syrup, acid added	Imitation coconut syrup	Imitation grape syrup	Imitation raspberry syrup	Aromatic eriodictyon syrup, N. F.	Syrup, U. S. P.
0	0	0.09	0.09	0.49	0.14	0.17	0.32	0.33	0.06	0.11	0.10	0.02	0.23	0.10	0	0.46	0.41
0.10	0.09	0.61	0.60	0.45	0.42	0.44	0.55	0.37	0.06	0.13	0.12	0.23	0.33	0.10	0.46	0.57	0.57
0.67	0.57	0.55	0.53	0.67	1.03	0.67	0.58	0.34	0.17	0.36	0.33	0.33	0.44	0.33	0.57	0.79	0.79
0.81	0.76	0.76	0.75	1.01	1.45	0.69	0.55	0.46	0.20	0.46	0.44	0.33	0.46	0.33	0.79	0.78	0.78
1.21	1.13	1.37	1.33	1.26	1.35	0.69	0.58	0.51	0.45	0.56	0.46	0.33	0.46	0.33	0.78	0.87	0.87
1.46	1.09	1.33	1.31	1.24	1.20	0.66	0.55	0.46	0.56	0.86	0.56	0.33	0.46	0.33	0.87	0.91	0.91
1.42	1.35	1.28	1.25	1.19	1.36	0.75	0.65	0.76	0.86	0.75	0.49	0.39	0.41	0.39	0.91	1.04	1.04
1.47	1.53	1.38	1.36	1.30	1.52	0.79	0.69	0.90	0.98	0.89	0.97	0.87	0.91	0.87	1.04	1.32	1.32
1.53	1.73	1.44	1.42	1.35	1.82	0.97	0.90	0.98	0.97	1.37	1.40	1.32	1.37	1.32	1.32	1.37	1.37
1.82	1.64	1.71	1.66	1.61	1.93	1.04	0.98	0.86	0.75	1.37	1.40	1.32	1.37	1.32	1.32	1.37	1.37
1.64	1.57	1.57	1.53	1.49	3.18	0.97	0.89	0.75	0.49	1.37	1.40	1.32	1.37	1.32	1.32	1.37	1.37
2.17	2.04	2.04	2.01	1.97	2.39	1.40	1.37	1.32	1.04	1.37	1.40	1.32	1.37	1.32	1.32	1.37	1.37

SUMMARY

A search of the literature revealed that some work had been done in the past on the evaluation of imitation flavors purchased from flavor manufacturers for disguising the taste of distasteful drugs. However, little or no work seemed to have been done to evaluate imitation flavors actually prepared in the laboratory from known materials. Therefore, an investigation was made to determine the relative efficiencies of some imitation flavored and official syrups as masking agents on the initial and obnoxious tastes of ammonium chloride.

It was found that some imitation flavored syrups were as good as the official syrups used, and in some instances superior.

The imitation fruit-flavored syrups, as such, were poor masking agents for the salty taste of ammonium chloride. The addition of citric acid to these syrups greatly enhanced their masking powers, and they were found to be among the best.

Raspberry syrup, N. F., imitation raspberry syrup, acid added, imitation wild cherry syrup, acid added, imitation grape syrup, acid added, cacao syrup, N. F., cherry syrup, N. F., imitation maple syrup, and glycyrrhiza syrup, U. S. P., were found to be the best for masking the initial salty taste of ammonium chloride. Not any in this group could be considered better than the other.

Imitation cream soda and imitation maple syrups were the most effective imitation flavored syrups for masking the obnoxious taste of ammonium chloride. Glycyrrhiza, raspberry, and cacao were the official syrups found as effective for masking the obnoxious taste.

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The Use of Some Imitation Flavors for Masking Distasteful Drugs. II. Quinine Hydrochloride*

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Cacao syrup, N. F., was the best of all vehicles at level of significance used for disguising the bitter taste of quinine. Most of the imitation flavored syrups were equally as effective disguising agents as the other official syrups used in this investigation. Of the imitation flavored syrups, coconut, raspberry, acid added, and wild cherry, with and without acid, were the best for masking the obnoxious taste of quinine. Equally effective of the official syrups were aromatic eriodictyon, cacao, and glycyrrhiza.

IN AN EARLIER paper (1) a study was made to determine the relative efficiencies of some imitation flavor concentrates, actually prepared from the basic ingredients, as masking agents on the initial salty and obnoxious tastes of ammonium chloride. These were compared with some of the official flavored syrups recognized as good masking agents for saline and bitter-tasting drugs. This investigation is a continuation of the previous work. Quinine hydrochloride was used as the distasteful or bitter-tasting drug.

HISTORY

Boothe and Kaufman (2) determined the masking effectiveness of 90 combinations of flavors for quinine sulfate. In this investigation a number of imitation flavor extracts purchased from flavor manufacturers, already prepared, were used. Root beer extract, peach, cherry, blackberry, grape, and pistachio were efficient imitation flavors for masking quinine. In addition, spirit of cinnamon, emulsions of orange and lemon oils, crème de menthe extract, oil of peppermint, and syrups of cacao and acacia were found efficient.

Purdum (3) found cacao, glycyrrhiza, and aromatic eriodictyon syrups of the official vehicles as excellent masking agents for quinine hydrochloride. Wright (4) reported aromatic eriodictyon and cacao syrups as efficient masking agents for bitter alkaloids, aromatic eriodictyon syrup being the better. Moreover, he claimed that raspberry syrup was even more efficient than these two.

Other pharmaceutical preparations reported as efficient masking agents for quinine are compound taraxicum elixir (5), tincture of cardamom (6), cinnamon, ginger, nutmeg, and acacia syrups (7), and aromatic eriodictyon elixir (8).

Munch and Ward (9) determined the masking action of certain inorganic salts on the bitter taste of strychnine. They found that the cation part of the salt was responsible for the masking effect on this drug, the magnesium cation being the most effective.

EXPERIMENTAL

The materials and formulas of imitation concentrates, preparation of syrups, taste test solutions and controls, and the method of evaluation of the vehicles used in this investigation were the same as described in a previous paper (1).

Concentrations of Solutions of Quinine Hydrochloride Used.—Fourteen concentrations of this drug were used the same as Purdum (3) employed except that in this work 4-cc. taste test samples were given to each subject. The normalities ranged from 0.0000029 to 0.00056, increasing by the ratio of 1:1.5. The concentrations were numbered from 1 to 14, respectively, in ascending order of normalities. The numbers of the solutions with the corresponding normalities and the concentration, in grams, of the drug contained in the 4-cc. samples used for the taste tests are given in Table I.

Results of Table II show that all syrups used were significantly better than water as masking agents for the taste of quinine hydrochloride, since the significance of difference was greater than 1.96. Cacao syrup, N. F., has the highest mean disguising potential of all syrups. Aromatic eriodictyon, N. F., and glycyrrhiza syrup, U. S. P., did not have as high mean disguising potentials as did imitation raspberry and coconut syrups. The addition of citric acid to the imitation fruit-flavored syrups did not appreciably increase their disguising potentials.

Table III shows that cacao syrup, N. F., was superior, at level of significance used, to all vehicles used for masking the bitter taste of quinine hydrochloride. This is indicated by the fact that a value of 1.96 or greater was found for all syrups with respect to this syrup. All vehicles from imitation coconut syrup to imitation butterscotch syrup, inclusive, were found to be of equal effectiveness for masking the bitter taste of this drug. This gives the physician a very wide range of vehicles from which to select, according to the likes of his patient. Imita-

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TABLE I.—FOURTEEN CONCENTRATIONS OF QUININE HYDROCHLORIDE USED

No. of Solution	Normality	Concentration of Drug in 4-Cc. Taste Test Sample, Gm.
1	0.000029	0.0000046
2	0.0000044	0.0000068
3	0.0000066	0.000011
4	0.000010	0.000016
5	0.000015	0.000024
6	0.000022	0.000035
7	0.000033	0.000052
8	0.000050	0.000078
9	0.000075	0.00012
10	0.00011	0.00018
11	0.00017	0.00027
12	0.00025	0.00040
13	0.00038	0.00060
14	0.00056	0.00090

tion grape syrup, acid added, and all syrups falling below it were found not to be significantly better than syrup, U. S. P.

It is seen in Table IV that all syrups mask the obnoxious bitter taste of quinine hydrochloride significantly better than water. Aromatic eriodictyon syrup had the highest mean obnoxious potential of all the syrups used. Imitation coconut and imitation wild cherry, acid added, had the next highest mean obnoxious potentials.

Table V shows that all vehicles from aromatic eriodictyon syrup, N. F., to and including glycyrrhiza syrup, U. S. P., were the best for masking the obnoxiousness of quinine hydrochloride. These were of equal effectiveness. All syrups below glycyrrhiza syrup, U. S. P., were inferior to those above, and were not significantly better than simple syrup.

TABLE II.—THE EFFECTIVENESS OF SOME IMITATION FLAVORED AND OFFICIAL SYRUPS ON THE DISGUISING POTENTIALS FOR QUININE HYDROCHLORIDE

Syrup	No. of Subjects	Mean Threshold in Water ± Standard Error $m_2 \pm \sigma_2$	Mean Threshold in Syrup ± Standard Error $m_1 \pm \sigma_1$	Mean Disguising Potential ± Standard Error $D \pm \sigma$	Significance of Difference
Cacao syrup, N. F.	15	6.07 ± 0.38	13.80 ± 0.20	7.73 ± 0.38	18.19
Imitation coconut syrup	15	5.80 ± 0.37	10.73 ± 0.40	4.93 ± 0.45	9.01
Imitation raspberry syrup	16	6.25 ± 0.42	11.06 ± 0.44	4.81 ± 0.37	7.95
Cherry syrup	16	6.56 ± 0.46	11.31 ± 0.43	4.75 ± 0.73	7.55
Aromatic eriodictyon syrup, N. F.	17	6.23 ± 0.26	10.88 ± 0.68	4.65 ± 0.53	6.59
Imitation raspberry syrup, acid added	15	6.00 ± 0.39	10.60 ± 0.51	4.60 ± 0.55	7.20
Imitation cream soda syrup	17	6.41 ± 0.43	10.94 ± 0.38	4.53 ± 0.50	7.81
Imitation wild cherry syrup	16	6.19 ± 0.48	10.69 ± 0.34	4.50 ± 0.38	7.65
Imitation maple syrup	17	6.35 ± 0.34	10.65 ± 0.40	4.30 ± 0.58	8.20
Glycyrrhiza syrup, U. S. P.	15	5.67 ± 0.31	9.93 ± 0.49	4.26 ± 0.60	7.50
Raspberry syrup, N. F.	15	6.20 ± 0.28	10.40 ± 0.48	4.20 ± 0.52	7.39
Imitation wild cherry syrup, acid added	15	5.87 ± 0.39	10.07 ± 0.60	4.20 ± 0.73	6.59
Imitation butterscotch syrup	19	6.16 ± 0.30	10.21 ± 0.41	4.05 ± 0.40	7.95
Imitation grape syrup, acid added	16	6.69 ± 0.28	10.44 ± 0.38	3.75 ± 0.34	7.81
Imitation root beer syrup	15	5.67 ± 0.32	9.13 ± 0.46	3.46 ± 0.49	6.06
Imitation grape syrup	15	6.53 ± 0.37	9.87 ± 0.44	3.34 ± 0.33	5.87
Syrup, U. S. P.	15	6.53 ± 0.36	9.60 ± 0.56	3.07 ± 0.41	4.65

TABLE IV.—THE EFFECTIVENESS OF SOME IMITATION FLAVORED AND OFFICIAL SYRUPS ON THE OBNOXIOUS POTENTIALS FOR QUININE HYDROCHLORIDE

Syrup	No. of Subjects	Mean Threshold in Water ± Standard Error $m_2 \pm \sigma_2$	Mean Threshold in Syrup ± Standard Error $m_1 \pm \sigma_1$	Mean Disguising Potential ± Standard Error $D \pm \sigma$	Significance of Difference
Aromatic eriodictyon syrup, N. F.	17	10.59 ± 0.26	13.82 ± 0.18	3.23 ± 0.28	10.00
Imitation coconut syrup	15	9.87 ± 0.58	13.00 ± 0.45	3.13 ± 0.31	4.30
Imitation wild cherry syrup, acid added	15	10.27 ± 0.34	13.27 ± 0.34	3.00 ± 0.50	6.25
Cacao syrup, N. F.	15	11.13 ± 0.57	14.00 ± 0.00	2.87 ± 0.46	5.09
Imitation raspberry syrup, acid added	15	10.73 ± 0.29	13.33 ± 0.26	2.60 ± 0.35	7.05
Imitation wild cherry syrup	16	10.81 ± 0.43	13.19 ± 0.25	2.38 ± 0.51	4.76
Glycyrrhiza syrup, U. S. P.	15	10.73 ± 0.54	13.06 ± 0.31	2.33 ± 0.60	3.85
Imitation grape syrup, acid added	16	11.00 ± 0.42	13.13 ± 0.28	2.13 ± 0.45	4.26
Imitation raspberry syrup	16	11.24 ± 0.47	13.06 ± 0.22	1.82 ± 0.43	3.50
Raspberry syrup, N. F.	15	11.33 ± 0.50	13.00 ± 0.25	1.67 ± 0.57	2.96
Imitation grape syrup	15	10.80 ± 0.50	12.40 ± 0.40	1.60 ± 0.33	2.51
Imitation butterscotch syrup	19	11.79 ± 0.22	13.37 ± 0.21	1.58 ± 0.30	5.15
Imitation root beer syrup	15	10.87 ± 0.53	12.27 ± 0.35	1.40 ± 0.64	2.20
Syrup, U. S. P.	15	10.80 ± 0.50	12.20 ± 0.48	1.40 ± 0.28	2.03
Cherry syrup, N. F.	16	11.88 ± 0.34	13.24 ± 0.26	1.36 ± 0.32	3.20
Imitation maple syrup	17	11.71 ± 0.33	13.06 ± 0.31	1.35 ± 0.39	3.79
Imitation cream soda syrup	17	11.71 ± 0.33	12.88 ± 0.26	1.17 ± 0.30	2.79

TABLE III.—THE SIGNIFICANCE OF DIFFERENCES BETWEEN THE MEAN DISGUSTING POTENTIALS FOR QUININE HYDROCHLORIDE WITH IMITATION FLAVORED AND OFFICIAL SYRUPS LISTED IN TABLE II

	Cacao	Imitation coconut	Imitation raspberry	Cherry	Aromatic eriodictyon	Imitation raspberry, acid added	Imitation cream soda	Imitation wild cherry	Imitation maple	Glycyrrhiza	Imitation rasberry	Imitation wild cherry, acid added	Imitation grape	Imitation root beer	Imitation grape, acid added	Imitation wild cherry, acid added	Imitation butter-scotch	Syrup, U. S. P.	Imitation grape
4.83					0.07	0.09	0.05	0.29	0.05		0.23	0.18							
5.50	0.21				0.16	0.15	0.30	0.38	0.13	0.08	0.72	0.55							
3.64	0.21				0.23	0.38	0.30	0.38	0.13	0.06	0.74	0.85	0.57						
4.75	0.41	0.07	0.11		0.44	0.42	0.35	0.46	0.11	0.29	1.03	1.07	0.92	0.47					
4.74	0.45	0.65	0.12		0.49	0.53	0.45	0.37	0.35	0.74	1.09	1.37	1.37	0.85	0.20				
5.16	0.60	0.32	0.25		0.60	0.44	0.37	0.82	0.81	0.74	1.43	1.39	1.72	1.26	0.60	0.51			
6.10	0.74	0.45	0.30		0.50	0.81	0.75	1.47	0.35	0.29	1.33	1.69	1.36						
4.97	0.85	0.59	0.48		0.91	1.31	1.28	1.68	1.09	0.74	1.63								
4.87	0.89	0.74	0.52		1.43	1.54	1.51	2.32	1.43	1.03									
5.43	1.06	0.77	0.61		1.63	1.97	1.98	2.55	4.75	1.33									
4.30	0.86	0.94	0.53		2.12	2.21	2.24			1.63									
6.69	1.46	0.74	0.84		2.36														
7.80	2.07	1.38	1.23																
6.90	2.19	2.08	1.47																
8.78	2.84	2.18	1.76																
8.33	3.05	2.94	2.01																
		3.10																	

TABLE V.—THE SIGNIFICANCE OF DIFFERENCES BETWEEN THE MEAN OBNOXIOUS POTENTIALS FOR QUININE HYDROCHLORIDE WITH IMITATION FLAVORED AND OFFICIAL SYRUPS LISTED IN TABLE IV

	Aromatic eriodictyon	Imitation coconut	Imitation wild cherry, acid added	Cacao	Imitation raspberry, acid added	Imitation wild cherry	Glycyrrhiza	Imitation grape, syrup, acid added	Imitation raspberry	Raspberry	Imitation grape	Imitation butter-scotch	Imitation root beer	Syrup, U. S. P.	Cherry	Imitation maple	Imitation cream soda
	0.14																
	0.40	0.29															
	0.67	0.54	0.19														
	1.40	1.21	0.66	0.47													
	1.47	1.32	0.87	0.71	0.36												
	1.36	1.24	0.86	0.71	0.39	0.00											
	2.08	1.89	1.29	1.15	0.83	0.37	0.27										
	2.76	2.55	1.79	1.69	1.42	0.85	0.69	0.50									
	2.48	2.31	1.75	1.65	1.41	0.94	0.81	0.64	0.24								
	3.70	3.41	2.33	2.23	2.08	1.28	1.06	0.95	0.41	0.01							
	3.94	3.54	2.40	2.31	2.18	1.32	1.10	1.00	0.45	0.01	0.04						
	2.61	2.49	1.96	1.86	1.65	1.19	1.06	0.94	0.55	0.32	0.27	0.25					
	4.57	4.21	2.81	2.72	2.67	1.69	1.41	1.37	0.82	0.43	0.45	0.43	0.06	0.09			
	4.25	3.94	2.74	2.65	2.58	1.67	1.41	1.38	0.85	0.47	0.51	0.48	0.06	0.10	0.02		
	3.92	3.64	2.62	2.53	2.40	1.61	1.38	1.32	0.83	0.46	0.49	0.46	0.07	0.55	0.41	0.36	
	4.91	4.55	3.10	3.04	3.04	2.01	1.70	1.75	1.23	0.77	0.94	0.91	0.32				

DISCUSSION

There were many syrups, both imitation flavored and official, that were of equal effectiveness for disguising the bitter taste of quinine hydrochloride. Cacao syrup, N. F., was the best at level of significance used. However, all vehicles, as a whole, did not appear as effective for masking quinine hydrochloride as for ammonium chloride.

The addition of acid to the imitation fruit-flavored syrups did not seem to improve the mean disguising and obnoxious potentials of these syrups. In the case of ammonium chloride (1), the addition of acid was beneficial.

It was also noticed that the taste of quinine hydrochloride in higher concentrations was even more obnoxious in vehicular solutions flavored with cherry syrup, N. F., imitation maple syrup, and imitation cream soda syrup, than with simple syrup, U. S. P. It is possible that these flavors augment the bitterness of quinine when higher concentrations are used.

SUMMARY

Cacao syrup, N. F., was the best of all vehicles at level of significance used for disguising the bitter taste of quinine hydrochloride. Most of the imitation flavored syrups were equally as effective disguising agents as the other official syrups used in this investigation. The addition of citric acid to the imitation fruit-flavored vehicles did not appear to increase the disguising potentials of these syrups.

Aromatic eriodictyon, cacao, and glycyrrhiza syrups were the best at level of significance used of the official syrups for disguising the obnoxious taste of quinine hydrochloride. Equally effective of the imitation syrups were coconut, wild cherry, with and without acid added, and raspberry syrup, acid added.

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The Synthesis of Certain Derivatives of Methionic Acid*

By RUE-CHENG SHU,† GLENN L. JENKINS, and JOHN E. CHRISTIAN

Six new derivatives of methionic acid possessing possible antibacterial value have been synthesized. These are: N,N′-bis-*o*-ethoxyphenyl methionamide; N,N′-bis-*m*-ethoxyphenyl methionamide; N,N′-bis-*p*-*n*-butylcarboxyphenyl methionamide; bis-α-naphthyl methionate; bis-β-naphthyl methionate; and bis-*o*-methoxyphenyl methionate.

METHIONIC ACID was discovered by Liebig (1) and has been investigated by Schroeter (2), Bauer (3), Elkas (4), and Ke (5). However, no pharmacological study of its derivatives has as yet been reported. In order that some additional methionic acid derivatives might be available for pharmacological testing, six new compounds have been made.

Attempts were made to synthesize the methionic acid derivatives containing the groups which are the components of many well-known drugs, such as β-diethylaminoethyl, β-ethoxyethyl, and β-ethoxyethylamino groups. However, these attempts did not meet with success by the procedures used.

Calcium methionate was available and was used as starting material to prepare the barium salt, which in turn was used to prepare the acid. Methionyl chloride was prepared from the acid and phosphorus pentachloride. The amines and phenols were then reacted with the acid chloride to form the desired anilides or esters (3, 6).

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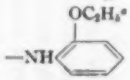
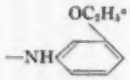
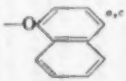
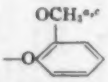
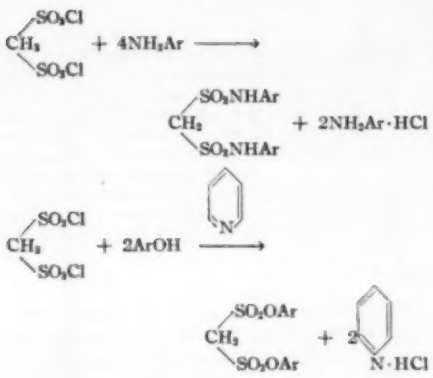
Presented to the Scientific Section, A. Ph. A., Atlantic City meeting, May, 1950.
† Purdue Research Foundation Fellow, 1948–1949.

TABLE I

R	Molecular Formula	Reactant[f]	Recrystallizing Solvent	M. p.[g]	Yield, %	Analysis (%) Nitrogen Calcd.	Nitrogen Found	Sulfur Calcd.	Sulfur Found
	$C_{17}H_{22}O_6N_2S_2$	*o*-Phenetidin, 0.2 mole	Alcohol-1,4-dioxane[d]	131–132	33.3	6.76	6.74	..	..
	$C_{17}H_{22}O_6N_2S_2$	*m*-Phenetidin, 0.2 mole	Alcohol	166.8–167.8	22.2	6.76	6.71	..	..
	$C_{22}H_{30}O_8N_2S_2$	*n*-Butyl *p*-aminobenzoate, 0.05 mole	Alcohol[d]	180.5–181	32.0	..	..	12.17	12.03
	$C_{21}H_{16}O_6S_2$	α-Naphthol, 0.1 mole	Alcohol-1,4-dioxane	136.8–137.4	13.1	..	..	14.95	14.95
	$C_{21}H_{16}O_6S_2$	β-Naphthol, 0.1 mole	Alcohol	235.1–236.1[e]	13.1	..	..	14.95	14.24
	$C_{15}H_{16}O_6S_2$	Guaiacol, 0.1 mole	Alcohol	99.6–100.4	16.0	..	..	16.49	16.25

[a] 0.05 Mole of methionyl chloride used. [b] 0.025 Mole of methionyl chloride and 8 cc. of pyridine used. [c] 15 cc. of pyridine used. [d] Decolorizing charcoal used. [e] Uncorrected. [f] Used to react with methionyl chloride. [g] With decomposition.



EXPERIMENTAL

Barium Methionate, Methionic Acid, Methionyl Chloride.—The procedures used were those of Bauer (3) and Ke (5). The following yields were obtained: 93.5% of the barium salt; 84% of the free acid; and 61% of the acid chloride.

N,N′-Bis-*p*-ethoxyphenyl Methionamide.—The following method used for preparation was different from that of Shroeter (2). Methionyl chloride, 10.6 Gm. (0.05 mole), was dissolved in 30 cc. of anhydrous benzene and the solution added dropwise with stirring to 200 cc. of anhydrous benzene containing 27.4 Gm. (0.2 mole) of *p*-phenetidin at 0°. A precipitate formed and the mixture was refluxed gently for one-half hour. After cooling, the mixture was filtered, and the solid obtained was washed with several portions of cold water. Decolorization of the remaining product from 1,4-dioxane by activated charcoal and then recrystallization from alcohol–1,4-dioxane mixture yielded 8.6 Gm. of white crystals (41.5% of the theoretical yield), m. p. 219.2 to 221° (uncorr.). Schroeter reported the melting point as 221°. No yield was reported.

Table I contains the compounds which were prepared by the procedure described above for the compound, N,N′-bis-*p*-ethoxyphenyl methionamide.

SUMMARY

1. Six new derivatives of methionic acid possessing possible medicinal value have been synthesized. These are: N,N′-bis-*o*-ethoxyphenyl methionamide; N,N′-bis-*m*-ethoxyphenyl methionamide; N,N′-bis-*p*-*n*-butylcarboxyphenyl methionamide; bis-*α*-naphthyl methionate; bis-*β*-naphthyl methionate; and bis-*o*-methoxyphenyl methionate.

2. Sufficient quantity of these compounds have been prepared for pharmacological testing.

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An Investigation of the Effect of Two Antihistaminic Agents on the Leukocytes of Peripheral Blood*

By H. ROBERT PATTERSON†,‡

An investigation of the effects of the administration of diphenhydramine and pyranisamine on the leukocytes of the peripheral blood in 16 human subjects was made. Administration of 75 mg. daily for fifty-six days produced no significant change in the blood picture, or prophylactic effect on the common cold.

SINCE the warning by the Council on Pharmacy and Chemistry of the American Medical Association of soporific side reactions of antihistamine drugs (1), further toxic manifestations have been reported. Cases where the drugs have affected the central nervous system, genitourinary, and hematopoietic systems have been noted (2). West and Peterson reported leukopenia in rats receiving Neo-Antergan and Pyribenzamine (3). Two reports of agranulocytosis in humans during therapy with antihistaminic agents of the ethylenediamine group have recently appeared in the literature (4, 5). It was the purpose of this investigation to evaluate and compare, under carefully controlled conditions, the effect of prophylactic (for the common cold) doses of an ethylenediamine derived, and a benzhydryl ether-type antihistaminic compound upon the leukocytes of peripheral blood.

* Received September 5, 1950, from the Department of Natural Sciences, San Jose State College, San Jose, Calif.
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‡ The author gratefully acknowledges the assistance given by Grace Mary Brown, Frank R. Passantino, and Clyde F. Wylie, technicians who were responsible for the determinations reported herein.

TABLE I

Patient	Normal AWBC[a]	Normal PMN%[b]	Final AWBC[c]	Final PMN%[c]
Group I, 75 Mg. Diphenhydramine Daily				
1	7,047	57.3	7,655	53.0
2	6,786	65.0	6,910	60.3
3	5,063	56.9	5,460	51.8
4	6,160	61.1	5,030	58.6
5	6,140	50.0	7,373	55.0
6	6,088	56.2	5,992	60.3
Av.	6,214	57.6	6,403	56.5
Group II, 75 Mg. Pyranisamine Daily				
1	6,934	57.4	7,573	58.9
2	7,214	57.78	7,058	59.9
3	7,777	59.5	7,355	59.08
4	6,967	59.0	7,068	57.91
5	7,008	54.64	7,166	58.75
6	7,157	55.37	7,636	60.41
7	6,983	56.07	7,346	56.75
8	7,098	58.07	7,236	59.08
9	7,235	56.5	7,190	57.91
10	7,631	60.35	7,441	59.75
Av.	7,200	57.46	7,307	58.85
Group III, Control				
1	7,490	59.08	7,440	62.41
2	6,436	62.58	7,256	62.75
3	6,963	62.08	6,820	62.41
4	6,283	55.16	6,923	54.50
5	6,013	61.75	7,033	61.25
6	6,741	59.25	7,040	61.33
7	7,193	67.66	7,425	64.41
Av.	6,731	61.08	7,134	61.29

[a] "Normal AWBC" is the average of six white counts taken during the two-week period prior to the institution of medication, and represents the normal count for the patient.
[b] "Normal PMN%" was also determined during the first two weeks of the experiment.
[c] The "Final AWBC and PMN%" is the average of counts taken during the seventh and eighth weeks of medication.

EXPERIMENTAL

Twenty-three subjects were selected from volunteer, healthy, adult college students, both male and female. These subjects were divided into three groups. Each group was assigned to one technician who was responsible for the determinations on each individual in his group during the entire course of the investigation, to avoid the introduction of errors due to individual variations in counting techniques.

From determinations made during the first two weeks of the investigation, the normal averages for each subject were estimated. At the beginning of the third week group I, consisting of six subjects, received 25 mg. of diphenhydramine hydrochloride three times daily, immediately before meals, for a total daily dose of 75 mg.; group II, consisting of ten subjects, received 25 mg. of pyranisamine maleate three times daily before meals for a total daily dose of 75 mg.; and group III, consisting of seven subjects, received no drug, serving as a control group.

Throughout the investigation, carefully controlled and standardized counting procedures were strictly adhered to by the technicians in the interest of consistent results. The technicians also recorded on the subject's chart any incident which might have an effect on the subject's leukocyte or differential count. Conditions such as colds, infections, allergies, and so forth, were recorded. The date of onset and the duration of such incidents were noted. Other medication taken by the subject during the course of the investigation was recorded.

The investigation was continued over a period of ten weeks, during which time the subjects were taking 75 mg. of the assigned drug daily continuously for a period of fifty-six days. The condensed results are presented in Table I. Values for lymphocytes, monocytes, eosinophils, basophils, and stabs have been omitted, as well as the individual graphs which show only a normal variation.

RESULTS AND CONCLUSIONS

Since variations of from 900 to 1,800 cells in the leukocyte count are not unusual (6), and variation of less than 10% in neutrophil values are not considered significant (7), it would appear that the peripheral blood picture was not altered in the subjects used in this investigation. It is interesting to note, however, that two subjects of group I, and four subjects of group II developed colds while receiving the antihistamines, while only one subject of the control group developed cold symptoms.

SUMMARY

There is no evidence from this investigation that a dose of 75 mg. daily for a period of fifty-six days of either pyranisamine or diphenhydramine had any adverse effect on the leukopoietic picture of 16 human subjects. It should be remembered, however, that this investigation was confined to a small number of cases, and larger scale investigations should be performed before universal conclusions are adopted.

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Stability of Penicillin-"Carbowax" Mixtures*,†

By RICHARD R. SHERWOOD‡ and ALBERT M. MATTOCKS§

"Carbowax" 1000[1] and polyethylene glycol 600 were found to inactivate penicillin. The inactivating effect could be removed in aqueous solution by adjustment of *p*H, but addition of alkali to molten anhydrous base did not have this stabilizing effect. Addition of BAL did not stabilize penicillin in "Carbowax"-polyethylene glycol mixtures.

ALTHOUGH penicillin is commonly used in ointments containing hydrocarbon or greasy bases several workers have demonstrated incomplete or very slow release of the antibiotic from these vehicles (1–3). Other workers have found good release from hydrophilic bases but very poor stability of the ointments, probably due to the high water content (2–11).

The physical properties of the "Carbowaxes" appear to meet the requirements for an ideal penicillin vehicle—"Carbowaxes" are anhydrous and yet water-soluble, and they may be mixed to obtain almost any desired consistency. Further, they have been reported to give excellent release of penicillin (2).

There have been conflicting reports on the stability of penicillin in "Carbowax." Andrews (12) prepared ointments which had an effective activity after three months' storage at 10°. Mohs (13) reported losses of penicillin in "Carbowax" which amounted to only 11 per cent at refrigerator temperature after seventy days. Meleney, *et al.* (14), reported 50 per cent losses after six to twelve weeks, and Macek, *et al.* (15), reported losses of 50 per cent after periods of storage varying from ten days to six months. These apparent differences are understandable, however, for there were in these studies many uncontrolled variables, such as temperature of manufacture, method of incorporating penicillin, conditions of storage, and presence of admixed substances.

Ferlauto and Clymer (16) compared the stability of penicillin in "Carbowaxes," propylene glycols, and glycerin, and found that very marked losses occurred with all three vehicles; almost complete inactivation resulting immediately with propylene glycol and glycerin, and a slower effect with "Carbowax."

No effort has been made to learn the nature of the inactivation of penicillin by "Carbowax," and the present investigation was undertaken to clarify the problem, and to learn whether some method may be found to remove the penicillin-inactivating effect of "Carbowax."

EXPERIMENTAL

Preliminary Tests with "Carbowax"

For this work only crystalline sodium penicillin-G was used.[2] It was planned to conduct preliminary experiments to give some insight into the effect of several variables on the stability of penicillin-"Carbowax" mixtures, and, finally, to test these effects in ointments prepared under sterile, controlled conditions. For the preliminary evaluations of penicillin potency the iodometric assay method of Alcino (17) was used, and for the final tests the more acceptable F. D. A. agar diffusion method (18) was used.

Charcoal Treatment of "Carbowax."—In view of the high adsorptive properties of activated charcoal, particularly for traces of organic impurities, it was decided to test whether charcoaling reduced the inactivating effect of "Carbowax" on penicillin. Ten grams of granular Nuchar-C was added to 100 Gm. of molten "Carbowax" 1000, and the mixture stirred for two hours at 50° and filtered while hot. Solutions of penicillin in "Carbowax" containing 1,500 units/Gm. were made by dissolving the penicillin in charcoaled and in untreated "Carbowax" at 60°. The mixtures were assayed iodometrically after one hour and at intervals during the ensuing five days' storage at room temperature. The results are shown in Table I.

TABLE I.—EFFECT OF CHARCOALING "CARBOWAX"

Ointment Base	—Per Cent of Original Potency, Days— 0	1	3	5
Charcoaled	21	19	10	7
Untreated	86	79	69	61

Effect of Heating on "Carbowax."—From the results of Table I it was apparent that the greatest loss of penicillin resulted within one hour after mixing, and since the charcoaled "Carbowax" had undergone a longer period of heating than the untreated sample, the latter being heated only for a period of one hour at 60° in order to dissolve the penicillin, an experiment was conducted to determine the effect of heating on the penicillin-inactivating properties of "Carbowax." Portions of "Carbowax" 1000 were heated in the oven at 60° for periods of one and one-half, three, four and one-

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† Presented to the Graduate School, Western Reserve University, by Richard R. Sherwood, in partial fulfillment of the requirements for the degree of Master of Science.
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§ Present address: American Pharmaceutical Association, 2215 Constitution Ave., Washington 7, D. C.
[1] "Carbowax" is a registered trade mark name of the Carbide and Carbon Chemicals Corporation, New York, N. Y.
[2] Furnished by Bristol Laboratories, Syracuse, N. Y.

half, six, and seven and one-half hours. The samples were then used to prepare penicillin ointments in the previous manner. The mixtures were assayed iodometrically twenty-four and seventy-two hours after preparation. Inasmuch as each sample required an additional hour of heating in order to dissolve the penicillin, this amount was added to the time stated above, and the ointment designated as untreated was actually heated for one hour. Results are shown in Table II. No difference in penicillin-inactivating effect beyond the limits of experimental error could be detected, the sample with the minimum of one hour of heating inactivating penicillin about as rapidly as those samples that were heated longer.

TABLE II.—EFFECT OF HEATING "CARBOWAX"

Time of Heating, Hr.	Per Cent of Original Potency, Hr. 24	72
1.0	79	69
2.5	82	70
4.0	81	70
5.5	79	67
7.0	72	63
8.5	66	55

Inactivating Effect of "Carbowax"-Aqueous Solution.—In order to eliminate the use of heat in the preparation of penicillin-"Carbowax" mixtures, it was decided to learn whether the "Carbowax" effect could be measured in aqueous solution, recognizing that only those inactivating agents which had a stronger effect on penicillin than water could be detected. Solutions of penicillin containing 2,500 units/cc. in water and in 25% aqueous solution of "Carbowax" were prepared and assayed at several intervals during storage in a refrigerator. Results are shown in Table III.

TABLE III.—STABILITY OF PENICILLIN IN AQUEOUS SOLUTION OF "CARBOWAX" 1000

Time of Storage, Days	—Per Cent of Original Potency— Penicillin in Distd. Water	Penicillin in 25% Soln. "Carbowax" 1000
1	99.3	97.7
2	98.7	94.0
4	98.3	93.3
6	98.3	93.3

Stability of Buffered Aqueous Solutions of Penicillin-"Carbowax."—A 5% aqueous solution of the "Carbowax" used in these experiments had a *p*H of 4.1, and it was decided to test the effect of buffering a "Carbowax"-penicillin solution at a *p*H known to enhance the stability of penicillin. Solutions containing 25% "Carbowax" were prepared as in the previous experiment, except that 1% phosphate buffer *p*H 6.2 replaced the distilled water. The stability of penicillin in these solutions is shown in Table IV.

Neutralization Experiments.—The improved stability obtained by buffering aqueous solutions of penicillin-"Carbowax" suggested the possibility of improving the anhydrous "Carbowax" by neutralizing free acids or removing them by extraction. It

TABLE IV.—STABILITY OF PENICILLIN IN BUFFERED SOLUTION OF "CARBOWAX" 1000

Sample	—Per Cent of Original— Potency, Days 1	2	5	7
Penicillin in 1% phosphate buffer *p*H 6.2	100	101	101	102
Penicillin in 1% phosphate buffer *p*H 6.2, containing 25% "Carbowax" 1000	100	101	100	102

was found that such salts as alkali citrates and tartrates were insoluble in the base, however, and this approach was discarded. Solutions of "Carbowax" in toluene and chloroform were treated with 30% aqueous sodium hydroxide both at room temperature and at reflux for varying periods of time, the immiscible liquids separated, and the "Carbowax" recovered by evaporation of the solvent under reduced pressure. Five per cent solutions of "Carbowax" so treated had *p*H values of 4.5 to 5.5. These products gave almost complete inactivation of penicillin solutions in one day, showing greater inactivating capacity than initially present.

In order to learn whether neutralization of the acidic material in "Carbowax" would remove the inactivating effect as well as buffering, the following experiment was conducted: samples of "Carbowax" were melted and maintained at a temperature just above melting while a saturated aqueous solution of sodium hydroxide was added dropwise. Samples were withdrawn at intervals and tested for *p*H, addition of alkali being continued until the *p*H of a 5% aqueous solution was about 6.5. These *p*H-adjusted samples were compared with the unadjusted material. Results are shown in Table V.

TABLE V.—STABILITY OF PENICILLIN IN SOLUTION OF NEUTRALIZED "CARBOWAX"

Sample	Per Cent of Original Potency, Days 2	6
25% Polyethylene glycol 600, *p*H 4.1[a]	48	20
25% Polyethylene glycol 600, *p*H 6.5	96	93
25% "Carbowax" 1000 *p*H 4.1	35	26
25% "Carbowax" 1000 *p*H 6.4	98	93

[a] *p*H refers to the *p*H of a 5% aqueous solution.

Tests on Ointment Formulations

Preliminary experiments indicated that for greatest stability, penicillin ointments should be prepared from "carbowax" which had been subjected to a minimum amount of heat, and that the "Carbowax" should be treated with alkali to bring the *p*H up to 6–6.5. It was recognized that the addition of water necessitated by the addition of alkali might affect penicillin stability, but it was decided to test this effect by a later series of experiments.

At the time these experiments were planned, there appeared in the literature a report on the enhancement of penicillin stability in solution by BAL

(British Anti-Lewisite), presumably through complexing metals contained in the mixtures (19), and it was decided to test whether BAL would increase the stability of "Carbowax"-penicillin ointments. Small amounts of BAL were incorporated in the ointment bases before adding the penicillin.

A mixture of eight parts "Carbowax" 1000 and two parts polyethylene glycol 600 was selected as a base suitable for ointments, and all subsequent ointments were prepared from this combination. Neutralization with alkali was carried out on the molten base as before. The same lots of "Carbowax" and of polyethylene glycol were used for all ointments.

Penicillin in a concentration of about 1,000 units/Gm. of ointment was incorporated into the various bases by mixing in a mortar at 40°. Sterile equipment and aseptic techniques were used throughout.

All potency tests on ointments were made by the F. D. A. method. The bases themselves were tested and found to exert no bacteriostatic action under the test conditions. The original assay on the ointment was carried out on the same day the ointment was prepared, this potency being taken as 100%. The ointments were stored at room temperature. Results are shown in Table VI.

TABLE VI.—STABILITY OF PENICILLIN IN "CARBOWAX"-POLYETHYLENE GLYCOL OINTMENT BASES

Ointment Base	Per Cent of Original Potency, Days 0	5	14	33	67
Untreated, *p*H 4.0[a]	100	74.5	56.4	33.3	6.9
Neutralized with NaOH, *p*H 6.5	100	79.6	61.8	49.2	15.2
0.6 mg. BAL/Gm. *p*H 4.0	100	74.1	49.1	17.8	4.6
0.3 mg. BAL/Gm. neutralized with NaOH, *p*H 6.5	100	85.2	50.5	35.7	6.0

[a] *p*H refers to *p*H of a 5% aqueous solution.

In order to test the effect of water contained in the ointment bases, the neutralized and untreated bases were dried at 50° in a vacuum system containing phosphorus pentoxide for forty-eight hours. Penicillin was incorporated as before, and the water content of the ointments was determined by the Karl Fischer method. Stabilities of these ointments are shown in Table VII.

TABLE VII.—STABILITY OF PENICILLIN IN DRIED "CARBOWAX"-POLYETHYLENE GLYCOL OINTMENT BASES

Ointment Base	Water Content, %	Per Cent of Original Potency, Days 0	7	39
Neutralized with NaOH, *p*H 6.5[a]	0.01	100	43.3	0.0
*p*H 3.8	0.06	100	65.9	9.4
*p*H 3.9	0.08	100	60.2	9.7
*p*H 3.8, 0.1 mg. BAL/Gm.	0.06	100	51.6	13.8
Neutralized with NaOH, *p*H 6.4, 0.1 mg. BAL/Gm.	0.01	100	62.0	28.5

[a] *p*H refers to *p*H of a 5% aqueous solution.

DISCUSSION AND CONCLUSIONS

These experiments confirm the fact that "Carbowax" 1000 and polyethylene glycol 600 have a powerful inactivating effect upon penicillin, at least in the samples tested. Further, it is shown by tests in aqueous solution that this effect is due chiefly to acidic nature which may be removed completely by addition of alkali or buffer. Treating the bases with charcoal or extracting them with aqueous alkali did not decrease their penicillin-inactivating power. Neutralization of the molten ointment base did not prove successful, improved stability by addition of alkali being shown only in aqueous solution. Inasmuch as it is known that a portion of the acidity of "Carbowax" and polyethylene glycol 600 is due to small amounts of phosphoric acid, and that addition of alkali would form alkali phosphates which exert buffer action, it appears that the penicillin in neutralized solution actually was protected by buffer. Thus, it seems likely that neutralization of molten base was not effective because no buffer effect was present in the nonaqueous medium.

SUMMARY

1. Samples of "Carbowax" and polyethylene glycol 600 were found to exert a powerful inactivating effect upon penicillin. This effect could be measured in aqueous solution.

2. The inactivating effect of these bases was removed in aqueous solution by adjustment of *p*H to 6–6.5. Addition of alkali to molten base did not stabilize ointments of penicillin which were relatively anhydrous.

3. Addition of BAL did not improve the stability of penicillin in "Carbowax"-polyethylene glycol 600.

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The Determination of Rutin in Tablets*

By EARL B. DECHENE

The colorimetric determination of aluminum by means of morin (5,7,2′,4′-tetrahydroxyflavonol) has furnished the basis for the colorimetric determination of flavonal compounds. A procedure based on this reaction has been developed for the determination of rutin in tablets. Paper partition chromatography has been employed to demonstrate the absence of any interfering flavonol compounds, such as quercetin, which might be present as breakdown products of rutin.

THE INCLUSION of rutin (5,7,3′,4′-tetrahydroxyflavonol-3-rhamnoglucoside) with other medicinal substances in tablets has led to this study of analytical methods for its determination in such preparations.

Porter, Brice, *et al.* (1), have reported a spectrophotometric method which can be applied only to preparations in which other components have a negligible ultraviolet absorption in comparison with rutin. Porter, Dickell, and Couch (2) state that a yellow color is formed in a solution of rutin on addition of aluminum chloride, and they used this reaction to determine the concentration of rutin in urine. Wender and Gage (6) have also used an aqueous solution of aluminum chloride both for quantitative leaching and determination of certain flavonol-3-glycosides from paper chromatograms. The use of morin (5,7,2′,4′-tetrahydroxyflavonol), which is structurally similar to rutin, has been described for the detection of certain metallic salts (3), and also for the colorimetric determination of aluminum (4). This type of reaction, therefore, suggested a basis for the colorimetric determination of rutin in tablets.

A modification of the method of Porter, *et al.* (2), was applied by us to the determination of rutin in tablets in which the precipitation of the aluminum-rutin complex with ammonium hydroxide and the re-solution of the precipitate in acetic acid was omitted. Though it has been reported (4) that the presence of more than traces of ionized salts depressed the color produced in the reaction with aluminum, we have found that the addition of potassium acetate to the reaction solution increased the color of the aluminum-rutin complex and rendered the test more sensitive. It was established in our experiments that a minimal concentration of aluminum chloride was required to obtain reproducible colors, and that these colors were not affected by the presence of ethyl alcohol in the reaction solutions.

However, since this reaction is reported to occur with quercetin (5,7,3′,4′-tetrahydroxyflavonol), and would appear to be general for the flavonols, it was necessary to show that the color produced by the tablet extracts was not due in part to quercetin or to some other breakdown product of rutin. Experiments were therefore carried out, using the paper partition chromatographic procedure of Wender and Gage (5), to show the presence or absence of quercetin in the tablet extracts.

EXPERIMENTAL

Reagents.—*Aqueous Aluminum Chloride Solution 0.1 M.*—Dissolve 2.41 Gm. of aluminum chloride ($AlCl_3 \cdot 6H_2O$, reagent grade) in 100 ml. of distilled water.

Aqueous Potassium Acetate Solution 1.0 M.—Dissolve 9.81 Gm. of potassium acetate ($KC_2H_3O_2$, reagent grade) in 100 ml. of distilled water.

Ethyl Alcohol.—SDAG No. 1-G. ethyl alcohol, refluxed ten hours with sodium hydroxide and zinc dust and distilled, was used in this work.

Alcoholic Solution of Quercetin.—A commercial sample of quercetin was air-dried at 125° to constant weight, and 0.050 Gm. dissolved in 50 ml. of the ethyl alcohol.

Standard Rutin Solution.—Commercial rutin was air-dried at 125° to constant weight, and the rutin content determined by the spectrophotometric procedure described by Porter, Brice, *et al.* (1). The standard solution was then made in alcohol to contain 50 μg./ml.

Color Reaction.—Aliquots of the standard rutin solution were transferred to test tubes, and alcohol was added to bring the volume in each tube to 5 ml. To each tube was added 3 ml. of aluminum chloride solution and 5 ml. of potassium acetate solution. After standing forty minutes, the transmittance of the yellow color was determined at a wave length of 415 mμ, as suggested by Porter, *et al.* (2), using a Coleman Spectrophotometer Model 11. A standard curve (Fig. 1) was drawn, using the average transmittance of three to four determinations of each concentration of rutin selected to suit the range of the instrument used.

The yellow color reached a maximum intensity in thirty minutes and appeared to be stable for several hours, although after ten hours a cloudiness appeared in the solutions, due to precipitation of aluminum hydroxide. The transmittance of the solutions was found to vary with the amount of aluminum chloride solution until a volume of 3 ml. was present. At this concentration the transmittance was constant and maximal, and showed no change as the concentration of aluminum chloride

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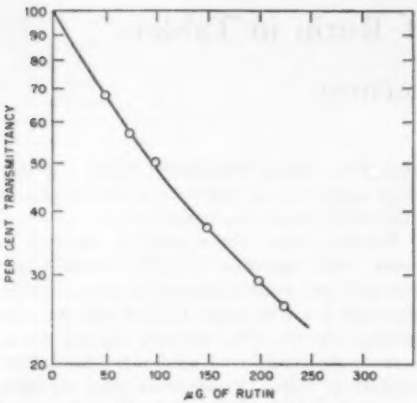

Fig. 1.—Standard curve for rutin-$AlCl_3$ color reaction.

was increased to 5 ml. In the same way, the effect of varying the concentration of alcohol from 3 to 7 ml. was found to have no effect on the color.

The Determination of Rutin in Tablets.—Rutin was determined in sugar-coated and uncoated compressed tablets containing: (*a*) 20 mg. rutin, 100 mg. aminophylline, 15 mg. phenobarbital; (*b*) 20 mg. rutin, 15 mg. mannitol hexanitrate, 20 mg. phenobarbital; (*c*) 15 mg. rutin, 15 mg. ascorbic acid, 15 mg. mannitol hexanitrate, and 15 mg. phenobarbital.

The average weight of one tablet was determined from the weight of 15 to 20 tablets, and 5 to 10 tablets were reduced to a fine powder. A weight of the powder, calculated to contain 15–20 mg. of rutin, was placed in a micro-Soxhlet apparatus and extracted for eight to ten hours with the SDAG No. 1-G. ethyl alcohol. The alcoholic extract was transferred to a 50-ml. volumetric flask, made to volume with the alcohol, and 15 ml. of this solution was transferred to a 50-ml. volumetric flask and made to volume with the same alcohol. The rutin content was determined in duplicate on 1-ml. aliquots of this solution in the manner described under "Color Reaction."

The results showed that 95–105% of the expected amount of rutin in the tablets was recovered by this method, and that no interference from other materials extracted from the tablets was present.

The Application of Paper Partition Chromatography to the Detection of Quercetin in the Tablets.—Rutin and quercetin, together and individually, were effectively separated in amounts from 4 to 50 μg. of each substance by means of paper partition chromatography. The solvent was a mixture of 4 parts of *n*-butanol, 5 parts of water, and 1 part of glacial acetic acid, as used by Wender and Gage (5) for the separation of flavonoid pigments. The apparatus described by Shephard (7), and Whatman No. 1 paper cut into strips 3.5 x 28 cm. were used in our experiments. The chromatograms were developed by allowing the solvent to rise on the paper strips, since it was found that this method produced more concentrated spots and reduced "tailing." The paper strips were air-dried and the boundaries of the fluorescent spots were located by spraying with aqueous aluminum chloride solution followed by aqueous potassium acetate solution, and examining the air-dried strips under a portable Cenco ultraviolet lamp. It was found possible by this procedure to show the presence of about 1 μg. of quercetin on the chromatograms, and this was considered to be the lower limit of detection. Without spraying, it was difficult to detect amounts of quercetin smaller than 4 μg. by means of the ultraviolet lamp. The presence of quercetin could be detected in commercial rutin when a sufficiently large sample was chromatographed.

The R_F values of rutin and quercetin shown by our chromatograms were higher than those reported (5), and were not constant, due to wide variations in room temperature during the twenty to twenty-four-hour period required to develop the chromatograms. Attempts were made to recover rutin quantitatively from the chromatograms by extracting sections of the paper strips containing the rutin with alcohol in a Soxhlet apparatus. The purity and the amounts of rutin recovered were determined by the spectrophotometric method (1) using a Beckman Model DU Spectrophotometer. It was found that rutin obtained in this way was of high purity but that it was difficult to extract quantitatively from the paper. The amounts of rutin recovered increased as the extraction time was increased, though a sixteen- to twenty-hour extraction period yielded only 65% of 50 μg. of rutin present on a chromatogram.

Suitable volumes of the alcoholic extracts of the tablets, containing 4–15 μg. of rutin, were chromatographed as described. Since only one fluorescent spot could be detected on these chromatograms with an R_F value in the range found for rutin, this was considered as evidence that no hydrolysis or breakdown of rutin had occurred in any of the tablet preparations examined. When quercetin was added to the alcoholic extracts of the tablets, it was separated on the chromatograms, indicating that there was no interference from other materials extracted from the tablets.

SUMMARY

1. Rutin in the presence of aluminum chloride produces a yellow color that is suitable for its quantitative determination under the conditions described.

2. Examination of various tablets by paper chromatography showed that rutin was extracted unchanged in the presence of aminophylline, ascorbic acid, barbiturates, and mannitol hexanitrate.

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Observations on the Exchange of Mercury between Phenylmercuric Chloride and Mercuric Chloride Using Mercury 203*,†

By JOHN M. CROSS and JOHN J. PINAJIAN‡, §

The exchange of mercury between phenylmercuric acetate and mercuric acetate using radioactive mercury 203 is reported. The reaction was found to come to equilibrium within the time required for mixing and separating the components (about one minute). This rapid exchange was found in the four solvents; glacial acetic acid, 50 per cent ethanol, water, and benzene. The exchange of mercury between phenylmercuric chloride and mercuric chloride was found to be slower, and several time-exchange curves in benzene solution were obtained. When the reactants were present in equimolar quantities (0.1 *M*), the half-time for the exchange in benzene was found to be thirty-seven and one-half minutes. Time *versus* exchange curves in dry benzene, wet benzene, and in acidified benzene at 24.8°, and in dry benzene at 44.8,° are given.

MERCURY COMPOUNDS, both inorganic and organic, have long been used as antiseptics and germicides, although the efficacy of the organo-mercury compounds as anti infectives has been questioned (1).

The most recent addition to the list of mercurial anti-infectives is a group of phenylmercuric salts. There is evidence to indicate that the phenylmercuric ion has a relatively high germicidal value against certain pathogenic bacteria, and a relatively low toxicity to human tissue (2). Among the compounds in this category used as germicides are phenylmercuric nitrate, phenylmercuric borate, phenylmercuric picrate, etc. (2). Phenylmercuric acetate is used at the present time as a weed-killer.

As a preliminary study of the action of these phenylmercuric salts, an investigation was undertaken to determine whether or not the mercury in these compounds could exchange readily with mercuric ion, and to study, if possible, the rate of this exchange. The evidence presented indicates that the mercury exchanges rapidly in the system phenylmercuric acetate–mercuric acetate in water, 50 per cent alcohol, glacial acetic acid, and in benzene. Phenylmercuric chloride and mercuric chloride exchange more slowly in benzene. The rate of this reaction is unaffected by the presence of water, but is accelerated by the addition of hydrochloric acid.

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EXPERIMENTAL

One gram of radioactive mercuric oxide was dissolved in 100 ml. of 30% acetic acid solution (this solution contained about 70 μc. of Hg^{203} per ml.).[1] This solution was used as a source of radioactive mercury in the exchange experiments in the acetate system.

Preliminary experiments indicated that it was most convenient to count so-called "infinitely" thick samples, that is, samples so thick that a further increase in thickness did not cause a change in counting rate. Experimentally, the minimum "infinite" thickness for Hg^{203} electrons in HgO was determined to be 79.5 mg./cm.[2] Consequently, all samples were counted as HgO and were prepared with slightly more than 80 mg. HgO/cm.[2] For collecting and counting samples of HgO, a cup was devised (3) which would insure uniform samples for counting.

The color of the HgO samples precipitated for counting was not uniform. Samples of HgO were therefore prepared ranging from yellow to dark red. The counting rates for all samples varied less than 2%. Slight variations in the color of the precipitates were therefore not considered significant.

Exchange of Hg in Phenylmercuric Acetate and Mercuric Acetate in Glacial Acetic Acid

Solution 1.—Phenylmercuric acetate (13.440 Gm.) was dissolved with the aid of gentle heat and brought to 200 ml. volume (0.2 *M* solution).

Solution 2.—Mercuric acetate (31.802 Gm.) was dissolved in glacial acetic acid, and 5 ml. of radioactive mercuric acetate solution added. The solution was brought to 500 ml. volume (0.2 *M* solution).

Twenty-five milliliters of solution 1 was added to 25 ml. of solution 2 in a separatory funnel, and immediately 50 ml. of benzene and 50 ml. of water were added. The funnel was shaken for ten seconds, and the benzene layer separated as quickly as possible. The benzene layer was washed with four 50-

[1] An absorption curve (aluminum absorbers) and a decay curve indicated a range of 80 mg./cm.[2] (about 0.30 Mev. for β) and a half-life of about fifty days. Both these values correspond roughly with the reported values for Hg^{203} (4, 5).

TABLE I.—EXCHANGE OF ϕHgOAc AND $Hg(OAc)_2$ IN GLACIAL ACETIC ACID[a]

Sample	Activity, Count/Min.	Ratio of Activity to That of Original $Hg(OAc)_2$ Sample	Apparent Exchange, %
HgO from ϕHgOAc following exchange expt. (sample 1)	622	0.494	98.9
HgO from ϕHgOAc following exchange expt. (sample 2)	624	0.495	99.1
Original $Hg(OAc)_2$ solution (solution 2)	1,258	...	...

[a] All measurements were made with an end-window Geiger-Mueller tube (4.0 mg./cm.^{2} mica window).

ml. portions of water. The benzene layer was collected and evaporated almost to dryness. The crystals of phenylmercuric acetate which formed had a melting point of 149.4 to 149.6°. These crystals were then refluxed with 10 ml. of ethyl alcohol and 20 ml. of concentrated HCl for four hours (6).

The acid solution was then evaporated to about 10 ml. to remove the alcohol, and 50 ml. of water was added. HgO was then precipitated by the addition of 5% NaOH solution.

The precipitate was transferred to a counting cup by suction filtration and washed with small portions of water, alcohol, and ether, and tamped down with a flattened glass rod to give a uniform and smooth deposit. The cup was placed in a vacuum desiccator to dry, and then weighed. It was then placed in an aluminum holder and counted.

A sample of the original radioactive mercuric acetate solution was precipitated with 5% NaOH and collected and counted in a similar manner. Counting data are shown in Table I.

In the system phenylmercuric acetate–mercuric acetate (glacial acetic acid solvent) the mercury exchanges completely within the time required for mixing and separating the components (about one minute).

Exchange of ϕHgOAc and $Hg(OAc)_2$ in 50% Alcohol Solution

The exchange experiments were run in a manner similar to the one described above. Equimolar solutions of ϕHgOAc and of $Hg(OAc)_2$ labeled with Hg^{203} were mixed and shaken for ten seconds, and the ϕHgOAc was extracted by shaking with benzene. In this instance the benzene and aqueous layers were extracted and purified by washing to determine the loss in activity of the $Hg(OAc)_2$, as well as the gain in activity of the ϕHgOAc. The results indicate that the exchange of Hg in the ϕHgOAc-$Hg(OAc)_2$ system is about complete within the one-minute period required for mixing and separating.

Exchange of ϕHgOAc and $Hg(OAc)_2$ in Water

Because of the limited solubility of ϕHgOAc in water, this exchange was run in 0.00425 *M* solution. The aqueous solutions were mixed and the ϕHgOAc was extracted with benzene. Complete exchange of the mercury was found within one minute.

Exchange of ϕHgOAc and $Hg(OAc)_2$ in Benzene

Because of the low solubility of $Hg(OAc)_2$ in benzene, the exchange was run in 0.015 *M* concentration. Again, complete exchange of the mercury was obtained within one minute.

Exchange of ϕHgCl and $HgCl_2$ in Benzene.—

ϕHgCl and $HgCl_2$ labeled with Hg^{203} were dissolved separately in benzene, and the benzene solutions mixed (each component in 0.006 *M* concentration), and shaken for ten seconds. The $HgCl_2$ was then removed as rapidly as possible by shaking with water. In the minute required for this procedure, about 20% of the activity had been transferred to the ϕHgCl.

Because of the somewhat slower exchange for ϕHgCl and $HgCl_2$, it was decided to study the exchange of this system. For this purpose, use was made of a glass-jacketed solution counter. This technique presents a much easier and more rapid method for studying the exchange, since the procedure of isolating the components in a dry form, mounting, etc., is avoided.

The counter, which had a constant background of about 40 c.p.m., gave reproducible results, and the active solutions could be washed off easily without leaving a residual count. The glass-jacketed counter was used in all of the following experiments.

Solutions of ϕHgCl and $HgCl_2$ labeled with Hg^{203} were dissolved separately in benzene to make 0.02 *M* solutions. Equal quantities of each solution were mixed (zero time) and 25-ml. portions were removed at various time intervals. These 25-ml. portions were shaken with 100 ml. of water for five seconds, and the benzene layer was separated and dried over anhydrous sodium sulfate. Fifteen milliliters was then placed in the counter, and the count, recorded. All samples counted were 0.01 *M* solutions of ϕHgCl in benzene.

It was necessary to increase the amount of radioactivity in these experiments, since the glass-jacketed counter records only the *gamma* radiation.

TABLE II.—EXCHANGE OF ϕHgCl AND $HgCl_2$ IN BENZENE AT 24.6°

Time	Activity of Separated ϕHgCl in Count/Min.	$A_\infty - A_t$[a]
2 min.	135	435
5 min.	171	406
10 min.	205	372
20 min.	261	316
30 min.	313	264
45 min.	386	191
90 min.	479	98
120 min.	554	23
9 hr.	567	...
15 hr.	587	...
53 hr.	543	...

[a] The specific activity of the $HgCl_2$ solution used indicated that complete exchange would result in a counting rate of 565 counts per minute for the separated ϕHgCl sample. This is in close agreement with the counting rates observed after nine to fifty-three hours. A_∞ was taken as 567 counts per minute.

Consequently the activity used in each exchange reaction was about 3.5 microcuries.

A preliminary test indicated that less than 4% of the original $HgCl_2$ remained in the benzene layer when this technique was followed.

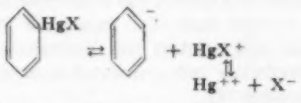
An example of the exchange study is shown in Table II. The procedure was followed under the following conditions:

a. In benzene saturated with water at 24.6°.
b. In dry benzene with 0.01 *M* HCl added at 24.6°.
c. In dry benzene at 24.6°.
d. In dry benzene at 44.6°.

The results of these exchanges are given in Figure 1.

DISCUSSION

The two most probable explanations for the rapid exchange of mercury between mercuric ion and phenylmercuric salts (especially the acetate) are: (*a*), the ionization of the phenylmercuric salt to give mercuric ion:

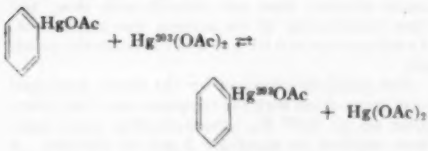


and (*b*), the association in solution of the phenylmercuric salt and the mercuric salt. If the former explanation is correct, the possibility is advanced that the efficacy of phenylmercuric salts as antiinfectives may be due to the liberation of a small concentration of mercuric ion.

SUMMARY

1. The exchange of Hg between phenylmercuric salts and mercuric salts has been investigated by the use of radioactive Hg^{203}.

2. The reaction:



was found to come to equilibrium within the time required to mix and separate the components (about one minute). This rapid exchange was found in the four solvents; glacial acetic acid, 50 per cent ethanol, water, and benzene. The exchange was so rapid that no study of the rate was made.

3. The exchange between phenylmercuric chloride and mercuric chloride was found to be slower, and several time-exchange curves in benzene were obtained.

4. When the reactants (phenylmercuric chloride and mercuric chloride) were present in equimolar concentrations (0.01 *M*) in the solvent benzene the following data were obtained:

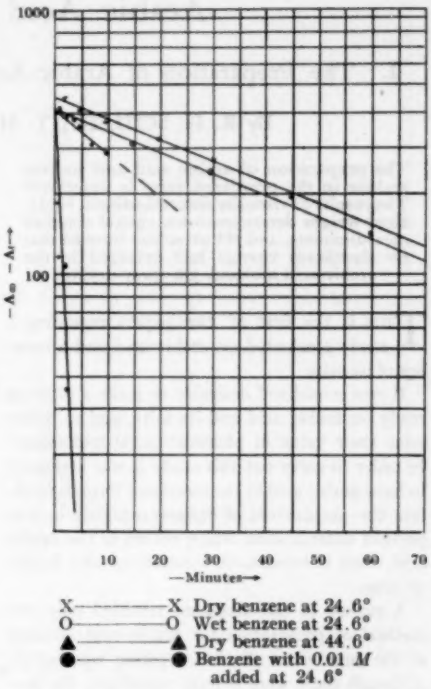


Fig. 1.—Exchange of Hg between phenylmercuric chloride and mercuric chloride in 0.01 *M* concentration in benzene.

Solvent	Temperature	Half-Time for Exchange, Min.
Dry benzene	24.6	37.5
Wet benzene	24.6	37.0
Dry benzene	44.6	18.0
Dry benzene +0.01 *M* HCl	24.6	0.8

The presence of water had little or no effect on the rate of the exchange. The presence of HCl greatly increased the rate of the exchange.

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Arabic Acid and Its Salts

I. The Preparation of Arabic Acid and Sodium Arabate Powder*,†

By R. H. SCHLEIF,‡ T. HIGUCHI, and L. W. BUSSE

The preparation of arabic acid and sodium arabate in the powdered form is described. The results of viscosity determinations, equivalent weight determinations, optical rotation measurements, and *p*H titrations showed that no significant change had occurred in the arabic acid molecule on spray-drying.

THIS is the first of two papers regarding a study conducted on arabic acid and a number of its salts.

It was considered desirable to make a physical study on arabic acid and its salts, and to determine their value in pharmaceutical technology. In order to carry out this study it was necessary to have arabic acid in the powdered form to facilitate the preparation of various solutions and to prevent deterioration which occurs in the arabic acid when it remains in solution for any length of time.

A survey of the literature revealed only one method of preparation for arabic acid solution at the time when the investigation was begun. Although there were several variations, the procedure in general was that as described by Saverborn (1–7). A solution (20 per cent) of gum arabic was acidified with hydrochloric acid and the acid was then precipitated from the solution with three volumes of alcohol. This procedure was repeated several times, and the solution was then dialyzed for one to two days. The ash content was reported to be reduced to 0.05 per cent (2). This method was tried, but it proved to be tedious, and only small quantities could be made at one time. The ash content also varied considerably from one batch to another.

Recently a method was reported which made use of ion-exchange resins to remove metallic ions. A column made up of Amberlite IR-100 was used. An ash content of 0.1 per cent was reported (8). This method was utilized in the present investigation to prepare the arabic acid solutions.

No successful procedure has been reported for the preparation of arabic acid in the solid state (5, 9–11). Conventional drying methods attempted in this investigation proved to be unsuccessful, but past experience with the spray-drier in this laboratory indicated that it might be a useful tool for the preparation of the arabic acid and sodium arabate powder.

Several methods of preparation of sodium arabate have been described (2, 4–6). One method involved the addition of sodium carbonate to a solution of acacia to precipitate the calcium ions. The solution was filtered and evaporated to dryness on a water bath (6). In another method an arabic acid solution was neutralized with sodium hydroxide and evaporated to dryness at 70° C. *in vacuo* (4).

EXPERIMENTAL PROCEDURE

Preparation of Arabic Acid.—A large column consisting of 6 lb. of Amberlite IR-120 (sodium-activated resin) was set up. The resin was changed to a hydrogen-activated resin by running a 10% solution of hydrochloric acid through the column and then rinsing with distilled water. Regeneration of the column was carried out in the same manner. Upon experimentation it was found that it was possible to put through 10 gal. of a 7% gum arabic solution before regeneration was necessary. In order to have a good margin of safety, only 5 gal. of gum arabic solution were put through each time, and then regeneration of the column was carried out. An ash content of 0.05% or less was set as the standard.

The solutions prepared by the above procedure were spray-dried with the temperature of the spray-drier set at 400° F. Approximately eight hours were required to spray-dry 5 gal. of solution. A fluffy, white, finely subdivided powder was obtained.

Preparation of Sodium Arabate.—Five-tenth normal sodium hydroxide solution was added to 5 gal. of arabic acid solution until *p*H 8 was reached. This solution was then spray-dried at 400° F. The powder obtained also had the same appearance as the arabic acid powder.

Physical Constants for Arabic Acid.—The following physical measurements were made to determine whether any changes had occurred in the arabic acid molecule

1. *Equivalent Weight*—Made with 0.1040 *N* sodium hydroxide, using bromothymol blue as the indicator.

2. *Viscosity Determinations*—Made with a Fenske viscosimeter in a constant temperature bath set at 30° C. Solutions were filtered in a sintered glass

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funnel before each determination. Specific gravity determinations were made with a Westphal balance.

3. *Specific Rotation*—Determined with a 2.001% solution of spray-dried arabic acid with a polarimeter at 24° C.

4. *pH Titration Measurements*—Made on arabic acid before and after spray-drying on a Beckman pH meter at 29° C. Titrations were made with 0.1040 *N* sodium hydroxide.

RESULTS AND DISCUSSION

From the results shown in Table I it may be seen that ash contents of 0.05% or less were obtained for all the batches of arabic acid solution made using the ion-exchange resin method. Acacia from three different 25-lb. lots was used.

TABLE I

Batches		Ash Content, %
L-I,	B-I	0.019
L-II,	B-II	0.05
	B-II	0.05
	B-III	0.05
L-III,	B-I	0.04
	B-II	0.03
	B-III	0.04
	B-IV	0.05

The results of several physical determinations showed that the spray-drying process did not change the physical and chemical properties of the arabic acid to any significant extent. Table II gives the results of equivalent weight determinations both before and after spray-drying. From these results it may be seen that no significant change occurred in the number of free carboxylic acid groups in the molecule. The results shown in the table are those from the batches prepared from the third lot of acacia.

TABLE II

Batch No.	Equivalent Wts. Before	After
1	1,279	1,279
2	1,215	1,291
3	1,299	1,300
4	1,177	1,300

The viscosity determinations which were made before and after spray-drying showed no significant change in viscosity by the spray-drying process. The results are shown in Figs. 1 and 2. Specific viscosity (η sp.) over concentration (c) was plotted against per cent concentration in order to more readily detect any difference in viscosity.

It may be noted from the curves in the two figures that there was a difference in viscosity for the arabic acid solutions prepared from the different lots of acacia. It was thought that this difference may have been due to either a difference in viscosity of the gum arabic used, or a difference in charge on the gum arabic molecules (12). Figure 3 shows that the difference was due to a difference in viscosity between the various lots of acacia, rather than a difference in charge on the molecule. This was shown by the fact that in the presence of sodium chloride, which neutralizes the charge, there was still a difference in viscosity between the various lots of gum arabic used.

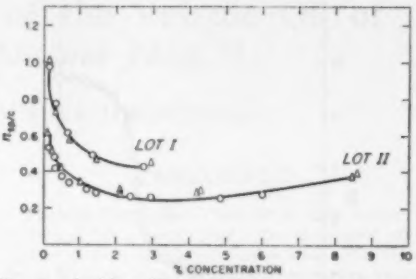

Fig. 1.—Arabic acid. ○, before spray-drying; △, after spray-drying.

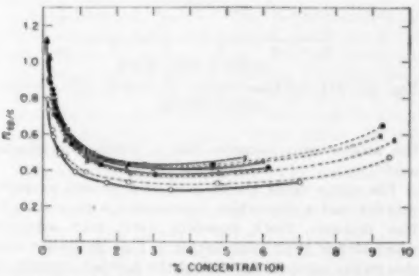

Fig. 2.—Arabic acid L-III. ——, before spray-drying; - - -, after spray-drying. ○, B-I; △, B-II; ●, B-III; ×, B-IV.

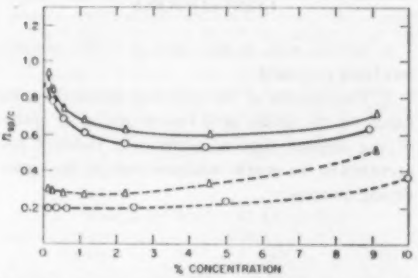

Fig. 3.—Gum arabic. ——, without 0.5 *M* NaCl; - - -, with 0.5 *M* NaCl; ○, L-II; △, L-III.

The specific rotation of a solution of arabic acid which had been spray-dried was determined to be −27.44°. The value given in the literature was −27.86° (13). This showed that hydrolysis did not occur in the molecule.

pH titration curves were made as a confirmation of results obtained by others, and as a check on the arabic acid prepared by the spray-drying process (4, 5). The curves given in Figs. 4 and 5 show no change, and also confirm the type of curve obtained by others. It may be seen from the curves that

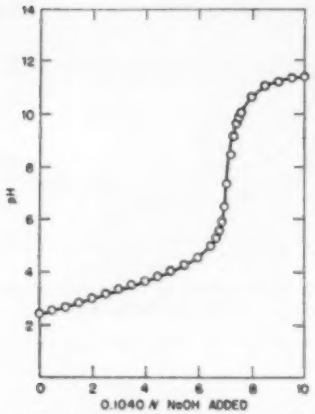

Fig. 4.—*p*H titration curve of arabic acid, before spray-drying.

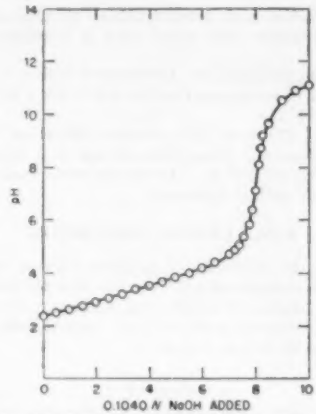

Fig. 5.—*p*H titration curve of arabic acid, spray-dried.

the arabic acid behaves like a strong monobasic acid.

The spray-dried arabic acid and sodium arabate powder had a fine white appearance with a starch-like texture. Both powders went into solution more slowly than gum arabic. This probably was due to the extreme fineness of the powder, causing it to clump more readily than the acacia in the presence of water.

CONCLUSIONS

1. Arabic acid, in the form of a dry powder, has been prepared.

2. The results of the physical determinations made on the arabic acid before and after spray-drying showed that no significant changes had occurred in the arabic acid molecule in the spray-drying process.

3. The spray-drying process is an excellent method for obtaining arabic acid and sodium arabate in large quantities in the dry powdered state.

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WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

Anthracene-2-sulfonic acid
2,3-Dihydroxyanthracene
Carbon tetraiodide
2,6-Epoxyhexane
3-Fluorophenylalanine
Camphane aldehyde
p-Methoxythiophenol
Maleimide
Ketomalic acid
3-Hydroxynorcholinic acid
Ionene
Lysergic acid
Neurine
Promulein
Protoporphyrin 111
Trypoflavin
Alloxazineadenine dinucleotide
D-Mannosamine
Capsaicin
Avidin

Chemical Investigation of the Volatile Oil of *Pycnanthemum pilosum* Nutt.*,†

By E. H. DJAO and ARTHUR E. SCHWARTING‡

The oil, distilled from plants cultivated in Nebraska and Iowa, contains pulegone and menthone as major constituents. Limonene and an alkali-soluble substance are present. A modified neutral sulfite assay was used in the determination of pulegone. Preliminary reduction experiments are reported.

PYCNANTHEMUM Michx. (1, 2), also *Koellia* Moench (3), is a genus of the *Labiatae*. There are 17 species, 12 being widely distributed in North America. *Pycnanthemum pilosum*, the hairy mountain mint, is of wide range, being found from Ontario to Pennsylvania, Georgia, Iowa, Kansas, and Arkansas.

Several investigators have examined the volatile oils of *Pycnanthemum lanceolatum* (4–8) and of *Pycnanthemum muticans* (9, 10), and reported the presence of pulegone and other constituents. Recently, Chilquist, Gilmour, and Jannke (11) reported the presence of pulegone in the oil of *Pycnanthemum pilosum*.

The present paper is based upon portions of a thesis, "An Investigation of *Pycnanthemum pilosum* and Its Volatile Oil," which reports histology and cultivation studies of the plant and chemical studies of the volatile oil.

The plants were established in the spring of 1947 at the Agricultural Campus of the University of Nebraska, Lincoln, Nebr., as well as at the Pellett Gardens, Atlantic, Iowa, and were harvested on July 16, 1948. Oils obtained in previous years, from a permanent planting at Atlantic, were also analyzed, and certain data are reported for several of these oils. At the time of harvest, the volatile oil content was 1.13 per cent for the whole above-ground plant, and 2.15 per cent for the leaf and flower. The oil was collected by steam distillation of the plant material, and an additional small amount of oil was obtained by cohobation of the aqueous distillate. The following experiments were carried out.

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† Abstracted from portions of a thesis submitted to the Graduate Faculty of the University of Nebraska by E. H. Djao in partial fulfillment of the requirements for the degree of Master of Science. Special acknowledgment is given to the Sioux Honey Association, under whose fellowship grant this work was completed, and to Frank Pellett, for his assistance in supplying plant material, and for initiating our interest in the subject of this investigation.
‡ Associate Professor of Pharmacognosy, College of Pharmacy, University of Nebraska, Lincoln. Present address: College of Pharmacy, University of Connecticut, New Haven.

EXPERIMENTAL

Physical Properties.—The oil is light yellow in color and has a sweet odor. The cohobated oil is yellow in color and has a similar odor. The cohobated oil shows a greenish fluorescence when viewed in reflected light. Physical constants are recorded in Table I.

Acid Value.—Negligible.

Saponification Value.—The determinations for both oils when refluxed for one or two hours showed no difference from the blank. Further determinations were carried out by refluxing for three hours. These results are reported in Table II. Since it is probable that some nonester substances in the oil might react with the alkali under reflux, the ester content reported may be doubtful.

Determination of Alcohols.—The samples were acetylated, and the saponification values of the acetylation products were determined in the usual manner. Results are reported in Table III.

Determination of Phenols.—The phenol contents were determined by extraction with an aqueous potassium hydroxide solution. The results were 3.8% for the oil, and 0% for the cohobated oil.

Determination of Aldehydes and Ketones.—The aldehydes and ketones are usually determined by the neutral sulfite or the bisulfite method. Each method required eight or more hours to complete the reaction. Neither gave consistent results.

Chilquist, Gilmour, and Jannke (11) determined the aldehydes and ketones of the 1945, 1946, and 1947 oils, and the 1947 cohobated oil by different methods. They reported results ranging from 7% to 55%. The most consistent results were obtained by the neutral sulfite method.

TABLE I.—PHYSICAL CONSTANTS

Sample	$d^{25°}$	n_D^{25}	$[\alpha]_D^{25}$
Oil	0.9248	1.4812	+14.2
Cohobated	0.9324	1.4843	+19.2

TABLE II.—SAPONIFICATION VALUE

Sample	Sapon. Value	Ester, Calculated as Menthyl Acetate, $C_{12}H_{20}O_2$, %
Oil	6.1	2.15
Cohobated oil	10.2	3.59

TABLE III.—ALCOHOL CONTENT

Sample	Sapon. Value, After Acetylation	Free Alcohol, Calculated as Menthol, % $C_{10}H_{19}OH$,
Oil	19.9	3.88
Cohobated oil	12.7	0.70

TABLE IV.—DETERMINATION OF ALDEHYDES AND KETONES

Sample	Method	1st Determination Carbonyl, %	1st Determination Time of Assay, Hr.	2nd Determination Carbonyl, %	2nd Determination Time of Assay, Hr.	Av. Carbonyl, %
Oil	Bisulfite	56	8	48	8	52
Oil	Neut. sulfite	79	8	78	8	78.5
Oil	Neut. sulfite with alcohol	76	1½	80	1½	78
Cohobated oil	Bisulfite	32	8	30	10	31
Cohobated oil	Neut. sulfite	90	8	90	8	90
Cohobated oil	Neut. sulfite with alcohol	90	1½	92	1½	91

Foote and Torres (10), in their work with *Pycnanthemum muticans*, reported an improved bisulfite method. They indicated that best results were obtained by using 10 cc. of ethyl alcohol and 10 cc. of oil and neutralizing the excess sulfur dioxide with sodium hydroxide.

In the present work, it was found that if 10 cc. of alcohol and 10 cc. of the oil is mixed and the usual neutral sulfite method is followed, the time of pulegone assay was lowered to about one and one-half hours, and the results obtained were in agreement with results obtained by the neutral sulfite method (12). The methods and results of assay are reported in Table IV.

The low results obtained with the bisulfite method are due to incomplete reaction. This was proved by treatment of the residual oil by the neutral sulfite with alcohol method. The residual oil gave 52% absorption, in one hour, by this method. The total absorption, calculated, was 77%. The residual oil of the cohobated oil from the bisulfite method gave 80% absorption, in one hour, by further treatment with neutral sulfite and alcohol. The total absorption, calculated, was 86%.

The refractive index of the regenerated products was determined. Results are tabulated in Table V.

Since some carbonyl compounds are known to be unreactive with sodium sulfite or bisulfite, they must be determined by other methods. The residual oil from the neutral sulfite method treated oil was further assayed by the hydroxylamine method (13). Duplicate experiments gave 89.43 and 87.80%, average 88.62%, calculated as menthone, $C_{10}H_{17}O$. This corresponds to 19.5% of the original oil.

Isolation of the Phenolic Substance.—Four hundred cubic centimeters of the oil was shaken with a dilute potassium hydroxide solution. The aqueous solution was separated from the insoluble oil, and washed with ether, acidified with hydrochloric acid to liberate the phenolic compound, and extracted with ether. The ether solution was dehydrated and evaporated. A small amount of yellow-brown viscid oil was obtained. Further purification by treating with different solvents did not change the appearance of this product. It was found to be soluble in sodium hydroxide solution but insoluble in sodium bicarbonate solution. The alcoholic solution of this product gave a green color with a ferric chloride solution. Attempts to prepare derivatives such as the α-naphthylurethane, phenylurethane, *p*-nitrobenzoate, and 3,5-dinitrobenzoate were unsuccessful.

Isolation and Identification of the Carbonyl Compound.—The oil, after removal of the alkali-soluble substance, was treated for eight hours with sodium sulfite solution. The aqueous solution of the sulfite addition compound was separated from the unabsorbed oil. The carbonyl compound was regenerated by addition of an excess of sodium hydroxide and was collected by steam distillation. The product separated from the distilled water was dried with anhydrous sodium sulfate. Boiling point 224°, with decomposition. Refractive index 1.4865 at 20°.

Forty-eight cubic centimeters of the product was subjected to fractional distillation in a 12-in. Widmer column under reduced pressure. The result is reported in Table VI. Constants are reported.

From the data of the distillation, the purity of the carbonyl compound can be established. The odor and physical properties of this compound correspond to that of pulegone. Derivatives were prepared. A 2,4-dinitrophenylhydrazone melted at 142–143°, reported for pulegone 142°. A semicarbazone, melted at 170–171°, reported for pulegone 174°.

Fractional Distillation of the Residual Oil.—The residual oil after the treatment with sodium sulfite solution was separated from the aqueous solution, washed with water, and dried with anhydrous sodium sulfate. Forty cubic centimeters was subjected to fractional distillation under reduced pressure. The results are shown in Table VII.

The odor and physical properties of fraction 1 suggest limonene. A bromine derivative was prepared by dissolving the sample in a mixture of amyl alcohol and ether and adding bromine dropwise until saturated. On evaporation of the ether a crystalline mass

TABLE V.—REFRACTIVE INDEX OF REGENERATED CARBONYL COMPOUNDS

Sample	Method	Refractive Index at 20°
Oil	Neutral sulfite	1.4853
Oil	Bisulfite	1.4857
Cohobated oil	Neutral sulfite	1.4850
Cohobated oil	Bisulfite	1.4848

TABLE VI.—FRACTIONAL DISTILLATION OF THE REGENERATED CARBONYL COMPOUND

Fraction No.	Temp. of of Dist. 2 Mm., °C.	Volume Collected	B. p. °C.	Refractive Index at 20°
1	52–57	1 cc.	220, dec.	1.4780
2	57–60	46 cc.	224, dec.	1.4865
3	60–57	A few drops	224, dec.	1.4879
Residue	...	A few drops	228–232 dec.	1.4960

TABLE VII.—FRACTIONAL DISTILLATION OF THE RESIDUAL OIL

Fraction No.	Temperature at 2 Mm., °C.	Vol. Collected, Cc.	B. p., °C.	Refractive Index at 20°
1	27–30	1	177	1.4675
2	43–49	2	204	1.4543
3	51–54	30	209	1.4528
4	56–51	2	215	1.4585
Residue	...	4	...	1.5100

was obtained; after repeated recrystallization from ether, ethyl acetate, and alcohol, the melting point was 98–100°. The literature gave the melting point for the tetrabromide of *d*-limonene 104–105°, of dipentene 125°. The bromine content of this derivative was determined by the bomb method (14), the results were 63.61 and 66.05%, average 64.83%. The theoretical bromine content for limonene tetrabromide is 70.80%, and dibromide, 54.42%.

The odor of fraction 2 was that of a mixture of menthone and limonene. Derivatives of menthone were prepared from this fraction. The 2,4 dinitrophenylhydrazone melted at 145–147°. The semicarbazone melted at 187–188°. The literature gave the melting points for the derivatives of menthone; 2,4-dinitrophenylhydrazone 146°, semicarbazone 189°.

The odor and physical properties of fraction 3 suggest menthone. The literature gave menthone, b. p. 209°, refractive index 1.450 to 1.451. A 2,4-dinitrophenylhydrazone prepared from this fraction melted at 145–146°; a semicarbazone melted at 185–187°.

Fraction 4 possessed an odor like menthol or menthone. Physical properties suggest menthol. Attempts to crystallize by cooling and attempted preparation of a phenylurethane, an α-naphthylurethane and a 3,5-dinitrobenzoate were unsuccessful, but a 2,4-dinitrophenylhydrazone was prepared, m. p. 144–145°, corresponding to menthone.

Preliminary Studies of Reduction of Pulegone, Methone, and the Oil.—The reduction of pulegone and menthone has been studied by numerous investigators (15). Catalytic hydrogenation of pulegone leads to a variety of products, depending upon the conditions used. In the presence of either colloidal palladium or platinum black, hydrogenation proceeds smoothly and produces *d*-menthone. Reduction of menthone with most reducing agents produces a mixture of stereoisomeric menthols in excellent yields.

In the present work, preliminary reduction experiments were carried out. Hydrogenation of the regenerated pulegone or the *Pycnanthemum pilosum* oil over Raney nickel or platinum black as a catalyst gave menthone in good yield. By reduction with sodium and alcohol, the crude menthone yielded a product which was probably a mixture of menthols, while the reduction of pulegone with the same reagents gave a dark brown viscid mass with an unpleasant odor. The separate experiments follow.

1. Pulegone Hydrogenation.—The hydrogenation of 10 cc. of regenerated pulegone in absolute alcohol over platinum black at a pressure range of 45–50 lb. yielded 5.5 cc. of a product distilling at 51–53°, at 1–2 mm. The distilled oil gave b. p. 208°, $n_D^{20°}$ 1.4560, $[\alpha]_D^{25}$ + 28°.

Hydrogenation of 10 cc. of the pulegone in absolute alcohol over Raney nickel catalyst at a pressure range of 43.5 to 50 lb. yielded 5.5 cc. of an oil. The oil gave b. p. 209°, $n_D^{20°}$ 1.4505, $[\alpha]_D^{25}$ + 30°.

2. Oil Hydrogenation.—One hundred cubic centimeters of the oil of *Pycnanthemum pilosum* dissolved in absolute alcohol over Raney nickel at a pressure range of 38–50 lb. yielded 82 cc. of an oil. The determination of the carbonyl compound content gave 0% by the modified neutral sulfite method and 96% by the hydroxylamine method. The oil distilled at 51–55°, at 2 mm., b. p. 209°, n_D^{20} 1.4495, $[\alpha]_D^{25}$ + 23. It yielded a 2,4-dinitrophenylhydrazone, m. p 144.5°, corresponding to menthone.

Fifty cubic centimeters of the crude oil obtained by the above process was dissolved in 100 cc. of absolute alcohol. Fifteen grams of sodium was added. The mixture, first cooled, was heated under reflux until the sodium was dissolved. The oil obtained by distillation at 4 mm. pressure was collected at 81–84°. The yield was 37 cc. The oil yielded a 3,5-dinitrobenzoate, m. p. 143–146°, and gave n_D^{20} 1.4591, $[\alpha]_D^{25}$ − 2° and b. p. 216°. The product began to congeal at 22°, and was complete at 15°, when melting, the temperature was constant at 17°. The product was probably a mixture of menthols. The yield was 57% of the whole oil.

SUMMARY

The volatile oil of *Pycnanthemum pilosum* Nutt. was investigated.

The plants, established in 1947, were harvested in 1948. The plant, at the time of harvest, contained 1.13 per cent of volatile oil. The leaves had a volatile oil content of 2.15 per cent.

The volatile oil contains 78 per cent of pulegone and 19.5 per cent of menthone, together with small amounts of limonene and an unidentified alkali-soluble substance. The presence of menthol is doubtful. A modified neutral sulfite method was used in the assay of pulegone.

Preliminary reduction experiments were carried out. Catalytic hydrogenation of regenerated pulegone or the crude oil gave menthone in good yield. Reduction of the crude menthone with sodium and alcohol gave a product, which was probably a mixture of menthols. The menthol yield obtained was 57 per cent of the whole oil.

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Quantitative Microbiological Determination of Penicillin*,†

By ROBERT W. SAGER‡ and LOUIS ARRIGONI

The production of acid as a metabolic product of microorganisms is commonly used as a measure of growth in microbiological procedures. A method for the quantitative determination of penicillin was therefore developed, in which growth inhibition, as measured by decreased acid production, was found to be a linear function of penicillin concentration. Nonpathogenic microorganisms were used in the tests, and the new method was found to compare favorably with standard, currently used procedures for the quantitative determination of penicillin.

THE FACT that so many methods and modifications exist for the quantitative microbiological determination of penicillin amply indicates that the ideal method has not yet evolved.

The most acceptable, currently used analytical methods are the Oxford cup (1) and the turbidimetric (2). In laboratories specializing in this type of analytical work, either of these procedures is satisfactory. However, for workers not accustomed to the particular details and equipment required, or for laboratories not performing such analyses routinely, either procedure leaves much to be desired.

The present study was undertaken in an attempt to develop a simple yet accurate penicillin assay requiring more readily available equipment, less experience by the technician and similar in fundamental approach to the microbiological assays of many vitamins and other biologically active compounds.

Since the production of acid as a metabolic product of microorganisms is commonly used as a measure of growth in microbiological procedures, experiments were undertaken to determine the sensitivity to penicillin of several nonpathogenic acid-producing microorganisms and an attempt was made to establish whether their growth inhibition, as measured by decreased acid production, was a linear function of increasing penicillin concentration.

EXPERIMENTAL

The lactic acid-producing microorganisms tested for sensitivity to penicillin were:

Streptococcus lactis—(American Type Culture Collection No. 7963).

Streptococcus fecalis DK—(obtained from A.C. Dahlberg and F. V. Kosikowsky, Cornell University, Ithaca, N. Y.).

Streptococcus fecalis R—(American Type Culture Collection No. 8043).

Lactobacillus arabinosus—(American Type Culture Collection No. 8014).

Lactobacillus casei—(American Type Culture Collection No. 7469).

Lactobacillus fermentum—(American Type Culture Collection No. 9338).

The medium adopted for growth of the microorganisms had the following composition:

Difco Yeast Extract[1]	2.0 Gm.
Difco Tryptone[1]	10.0 Gm.
Dextrose	20.0 Gm.
Salt Solution A	10.0 ml.
Salt Solution B	10.0 ml.
Distilled water, to make	1,000.0 ml.

Salt solution A consisted of 25.0 Gm. each of K_2HPO_4 and KH_2PO_4 in 250.0 ml. of aqueous solution. Salt solution B consisted of $MgSO_4 \cdot 7H_2O$, 10.0 Gm.; NaCl, 0.5 Gm.; $FeSO_4 \cdot 7H_2O$, 0.5 Gm.; $MnSO_4 \cdot 4H_2O$, 0.5 Gm.; distilled water 250.0 ml.

Before making the solution to its final volume, the *p*H was adjusted to 7.0 with sodium hydroxide.

In the preparation of the inoculum it is necessary to activate the microorganisms, since it has previously been shown that maximum susceptibility to penicillin is reached when rapid multiplication of microorganisms takes place. In order to accomplish this, transfers were made from the stock cultures to new culture tubes containing sterile nutrient medium, and incubated for twenty-four hours at 37°. In each case, cells from the fresh culture were then transferred to a tube containing approximately 10 ml. of the sterile nutrient broth to be used in the assay, and incubated for twenty-four hours at 37°. A loopful of this broth culture was then transferred to another tube containing approximately 10 ml. of the sterile nutrient broth, and incubated for twenty-four hours at 37°. This latter broth culture was then used as the inoculum in the determination of the extent of growth of the microorganisms in the presence of graded concentrations of penicillin.

In order to determine the concentration of penicillin necessary to inhibit the growth of each microorganism, as evidenced by decreased acid production, experiments were undertaken using a series of penicillin concentrations varying from 0.1 to 1.0 unit per ml. These penicillin solutions were prepared by the addition of a weighed amount of pure crystalline potassium penicillin-G to sterile phosphate buffer solution, *p*H 7.0 (one unit of penicillin is equivalent to 0.6 μg. of pure crystalline potassium penicillin-G).

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[1] Difco Laboratories, Inc., Detroit, Mich.

Equal volumes of penicillin solutions of graded potency (0.1 to 1.0 unit per ml.) and sterile broth were added to test tubes, along with one drop of an inoculum of the test microorganism, prepared as previously described. After a period of incubation suitable to the microorganism used, as indicated in Table I, the contents of the tubes were titrated to *p*H 7.0 with 0.05 *N* sodium hydroxide. Ranges of penicillin concentration were then chosen which showed a satisfactory gradient of decreasing acid production with the test microorganism.

It was observed that the degree of inhibition with increasing concentrations of penicillin tended to deviate from the linear as complete inhibition was approached. Since the most valid portion of a curve is in the range which shows the greatest change between successive increments, a series of penicillin concentrations was chosen for each microorganism which varied from slight inhibition to somewhat less than complete inhibition.

TABLE I.—INCUBATION TIMES FOR VARIOUS MICROORGANISMS

Microorganism	Hr.
Streptococcus lactis	24
Streptococcus fecalis DK	24
Streptococcus fecalis R	24
Lactobacillus arabinosus	72
Lactobacillus casei	72
Lactobacillus fermentum	48

PROCEDURE

Since all the microorganisms tested in this investigation were found to grow well in the nutrient broth previously described, the only variables in determinations with the different microorganisms were the concentrations of penicillin required to produce inhibition of growth and the incubation time.

To each of six test tubes, 22 x 175 mm., was added exactly 10.0 ml. of the nutrient broth, then in succession 10.0, 8.0, 6.0, 4.0, 2.0, and 0.0 ml. of 0.02 *M* phosphate buffer solution, *p*H 7.0, as indicated in Table II. The tubes were stoppered with nonabsorbent cotton and sterilized in the autoclave for twenty minutes at 15 lb. pressure (120°).

TABLE II.—PREPARATION OF TUBES FOR STANDARD CURVE

Tube	Nutrient Broth, Ml.	Phosphate Buffer, Ml.	Standard Penicillin, Ml.
1	10.0	10.0	0.0
2	10.0	8.0	2.0
3	10.0	6.0	4.0
4	10.0	4.0	6.0
5	10.0	2.0	8.0
6	10.0	0.0	10.0

A standard penicillin solution, whose concentration was dependent upon the susceptibility of the microorganism (Table III), was prepared in sterile 0.02 *M* phosphate buffer solution and added to the tubes in amounts of 0.0, 2.0, 4.0, 6.0, 8.0, and 10.0 ml., respectively, as indicated in Table II. The final volume in all tubes was 20.0 ml.

TABLE III.—PENICILLIN SOLUTIONS FOR DETERMINATION OF STANDARD CURVES

Microorganism	Standard Soln., Units/Ml.
Streptococcus lactis	0.4
Streptococcus fecalis DK	2.0
Streptococcus fecalis R	0.5
Lactobacillus arabinosus	0.5
Lactobacillus casei	0.5
Lactobacillus fermentum	0.2

One drop of the inoculum, prepared as previously outlined, was added to each tube, the contents mixed by rotation and incubated at 37° for the appropriate time, depending on the test microorganism, as indicated in Table I.

The contents of each tube were then transferred to a suitable container, rinsed with approximately 50

TABLE IV.—COMPARATIVE ANALYSES

Sample	Actual Concn., Units/Ml.	Oxford Cup		Turbidimetric		*S. Fecalis* DK	
		Found	Deviation, %	Found	Deviation, %	Found	Deviation, %
1	1.1	1.01	−8.2	0.96	−12.7	1.06	−3.6
2	9.7	10.10	+4.1	9.63	−0.7	9.40	−3.1
3	79.3	66.3	−16.4	96.3	+21.4	81.0	+2.1
4	923.0	770.0	−16.6	963.0	+4.3	990.0	+7.3
5	86.8	113.0	+30.2	81.0	−6.7	92.0	+6.0
6	78.8	80.0	+1.5	79.5	+0.9	91.0	+15.5
7	92.0	103.5	+12.5	76.5	−16.8	94.0	+2.2
8	111.7	115.0	+3.0	96.0	−14.1	118.0	+5.6
9	94.6	110.0	+16.3	79.5	−16.0	94.0	−0.6
10	77.0	91.0	+18.2	83.0	+7.8	80.0	+3.9
11	100.0	124.0	+24.0	...	...	90.0	−10.0
12	114.0	120.0	+5.3	98.0	−14.0	102.0	−10.5
13	142.0	130.0	−8.5	...	...	122.0	−14.1
14	112.0	112.0	0.0	100.0	−10.7	100.0	−10.7
15	133.0	130.0	−2.3	103.0	−22.6	114.0	−14.3
16	92.0	85.0	−7.6	105.0	+14.1	86.0	−6.5
17	113.0	128.0	+13.3	...	...	101.0	−10.6
18	70.0	78.0	+11.4	...	...	79.0	+12.9
			Mean 11.1		Mean 11.6		Mean 7.8

ml. of water, and titrated to *p*H 7.0 with 0.05 *N* sodium hydroxide, using a *p*H meter.

Each of the above levels of penicillin was determined in triplicate, the titration figures averaged, and a curve was prepared with penicillin concentrations in units per ml. as the abscissa and ml. of 0.05 *N* sodium hydroxide as the ordinate.

Tubes for unknown samples were prepared, in triplicate, containing 10.0 ml. of the sterile nutrient broth and 10.0 ml. of the proper dilution of the unknown penicillin preparation. Unless the approximate potency of the unknown preparation is known to the analyst, it is necessary to choose a random series of dilutions to test simultaneously with the standard series. Such a series of random dilutions may contain one or more dilutions which will fall upon the valid portion of the standard curve, or may only indicate the extent of the dilution necessary, in which case the determination, including the standard series, must be repeated with the appropriate dilution of the unknown penicillin preparation.

Since the standard curves are based upon units of penicillin per ml. of total solution, and half the volume in each tube consists of nutrient broth, the actual concentration of the added unknown penicillin solution in units per ml. will obviously be twice that of the total solution. Thus the unitage of the diluted unknown solution may be calculated by multiplying the concentration obtained from the standard curve by two.

COMPARATIVE ANALYSES

In the proper evaluation of new analytical procedures it is necessary to compare them with the most acceptable, currently used analytical methods under identical conditions. Accordingly, comparative analyses on identical samples were performed with the proposed, the Oxford cup (1), and the turbidimetric procedure (2).

Streptococcus fecalis DK was chosen as the test microorganism in the proposed method, since it had exhibited the most consistently reproducible standard curves. The results of these comparative analyses are shown in Table IV.

SUMMARY

Six nonpathogenic, acid-producing microorganisms, *S. lactis*, *S. fecalis DK*, *S. fecalis R*, *L. arabinosus*, *L. casei*, and *L. fermentum*, were tested for their susceptibility to penicillin.

Ranges of penicillin concentration were established for each microorganism through which the inhibition of growth, as measured by decreased acid production, remained linear.

The linearity of decreasing acid production with increasing penicillin concentration was adapted to a quantitative procedure.

Streptococcus fecalis DK was chosen as the test microorganism in the proposed method, since it had exhibited the most consistently reproducible standard curves.

The new method was found to compare favorably with the Oxford cup and turbidimetric microbiological procedures which are currently being used.

Comparative results obtained from analyses of 18 penicillin solutions of unknown strength showed an average error of 7.8 per cent with the proposed method, 11.1 per cent with the Oxford cup, and 11.6 per cent with the turbidimetric method.

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WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

p-Hydroxyhippuric acid
Uramildiacetic acid
Ferritin
Toxisterol
L-Xylulose
Hexokinase
3-Hydroxycholinic acid
Thymidylic acid
Isolysergic acid
Trimethyl-(*p*-aminophenyl)ammonium chloride
Indigo brown
Vulbocapnine
Primula acid
Phenamidine
Copper pyridine thiocyanate
Diadzin
Quercitagetin
Myricetin

Use of Infrared Spectra in the Qualitative and Quantitative Determination of Alkaloids*

By GEORGE B. PLEAT,† JOHN H. HARLEY,‡ and STEPHEN E. WIBERLEY§

The infrared spectra of 22 alkaloids are reported in the region of 3 to 14 μ. Each alkaloid examined has a unique spectrum, and it is possible to differentiate not only between individual alkaloids but also between a free alkaloid base and its salt. Using the known infrared spectra, qualitative analysis in a mixture of alkaloids is feasible. Also, a wave length can be selected at which other alkaloids do not absorb, and a quantitative analysis for the desired alkaloid can be made. Using suitable solvents, a linear relationship is indicated when the absorbancy values obtained are plotted *vs.* the concentration over a range of concentration from approximately 1 to 100 mg./ml. The accuracy of the analysis is dependent upon the amount of sample available ranging from less than 1 per cent relative error for large amounts to almost 20 per cent for amounts in the region of 1 mg.

THE ALKALOIDS have been the subject of many investigations dealing with their isolation, identification, and their physiological properties. The isolation of an alkaloid involves, first, the extraction of the total alkaloids from the parent substance, followed by a series of extractions with organic solvents to separate the constituents. Such treatment usually yields groups of similar alkaloids rather than the individual compounds. Since the chemical and physical properties of the alkaloids are so similar, the final analysis is therefore usually reported in terms of the various groups.

For the identification of an alkaloid the usual methods adopted for organic compounds, i. e., the determination of physical constants such as the melting point for the free alkaloid and for one or more of its derivatives, are used. Color reactions are frequently employed, but these are often unsatisfactory due to impurities, and when they have to be used, are usually applied, side by side, to the substance under examination, and to a pure sample of the alkaloid.

It appears, therefore, that any method of analysis which would lead to the rapid identification and estimation of alkaloids singly, or in the presence of one another, would be of considerable value in pharmaceutical, and possibly forensic, chemistry. The most obvious approach is the use of infrared spectra which are specific for individual compounds.

Spence (1) was the first to demonstrate that such an investigation had definite possibilities. Using a spectrophotometer of special design equipped with a rock salt prism, he was able to show absorption bands for compounds in the pyridine, quinoline, tropine, and isoquinoline groups. Some time later, Bell (2) investigated the infrared absorption spectra in the range of 1 to 12 μ of the tropine derivatives. O'Byrne (3) has reported the infrared absorption of certain constituents in the pyridine, glyoxaline, and quinuclidine groups in the same region of the spectrum. In this article the presence of such groups as C-N and C_4H_5 was corroborated by their respective bands. Recently Blout and Fields (4) have shown the value of infrared spectra in characterizing purines and pyrimidines. Two of the alkaloids, caffeine and theobromine, run by these investigators were also measured in this study.

In view of the work of these investigators, it seemed logical that for qualitative work any alkaloid could be distinguished by its characteristic absorption bands. No application to the quantitative estimation of the alkaloids by infrared spectra has been found in the literature.

EXPERIMENTAL

The alkaloids used in this work were U. S. P. grade and were obtained from both the Mallinckrodt Chemical Company and Eimer and Amend. As a check on their purity, melting point determinations were run on the solids and compared with accepted literature values. All of the alkaloids utilized in this study checked within ±1° of the reported literature value. In the case of the liquids, the compounds were redistilled and a constant boiling fraction was utilized for the infrared absorption spectra. Further efforts were not directed toward detailed purification of these compounds because any commercial application of this method would involve ordinary U. S. P. grade drugs.

The measurements were made on a Perkin-Elmer Model 12B infrared recording spectrometer equipped with a sodium chloride prism. The prism was calibrated using ammonia, carbon dioxide, and atmospheric water bands.

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§ The authors are indebted to John Larner, who ran the melting point determinations and aided in preparation of the figures.

Qualitative Applications.—The spectra of the alkaloids available were measured over a range of about 3 μ (3,600 cm.$^{-1}$) to 15 μ (700 cm.$^{-1}$).

All of the alkaloids measured, with the exception of two (nicotine and piperidine), are solids, some of these being in the form of the basic amine, and others as the hydrobromide or the hydrochloride. In working with the solid alkaloids, a sample was prepared by grinding a small amount with a mortar and pestle to as fine a powder as possible. The fine powder was then mulled with a few drops of Nujol, a straight chain aliphatic hydrocarbon, and a small amount placed in an adjustable cell fitted with a lead spacer and sodium chloride windows.

It is necessary to select for the particular compound an optimum cell thickness which will give sufficient absorption and yet allow good resolution. A 0.025-mm. spacer was found satisfactory in the case of all of the alkaloids studied.

The Nujol absorbs strongly in the region of the characteristic C-H frequencies, which occur between 3.20 and 3.57 μ, and obscures any absorption in this region due to the alkaloid. As most of the C-H bands occur in the higher frequency region, and as most organic compounds contain the C-H linkage, the use of such bands as a means of qualitative and quantitative estimation would be impractical. Other weak Nujol absorption bands which might interfere are the following: 3.67, 6.82, 7.27, 7.67, 8.64, 10.24, 10.87, 11.22, and 13.82 μ.

The absorption bands in the region from 3 to 14 μ of all the alkaloids investigated are shown in the bar chart of Fig. 1. It can be seen that the alkaloids have been listed by groups; pilocarpine hydrochloride being in the glyoxaline group, with the other groups following in alphabetical order: isoquinoline, purine, pyridine, quinoline, and tropine groups. At the end of these groups is listed one unclassified alkaloid. Quinoline and pyridine have been included for comparison with other members of their group. It can be seen that, although in the farther infrared there is a trend toward similarity in one particular group, it is difficult to ascribe any bands peculiar to one group. Also, it will be noted that there are no identical absorption spectra for any of the alkaloids listed. Thus each absorption spectrum is characteristic of the compound and serves as a means of identification.

Several assignments can be made from Fig. 1. The band found at approximately 6.3 μ which occurs in several of the alkaloids studied can be ascribed to the double-bond stretching of the benzene nucleus. Although the aliphatic carbon hydrogen stretching frequencies have not been obtained for the solids due to the interference of the mulling agent, Nujol, previously discussed, in the liquids nicotine, and piperidine these bands appear at approximately 3.6 μ. The band found at approximately 3.4 μ in nicotine, pyridine, and quinoline can be attributed to the aromatic C-H stretching vibration. The band at 3.1 μ in piperidine can be assigned to the N-H group.

The absorption bands shown in Fig. 1 in the region above 8.5 μ cannot be assigned to characteristic group frequencies. However, these frequencies which are due to the larger groups in the molecule are valuable for qualitative and quantitative analysis.

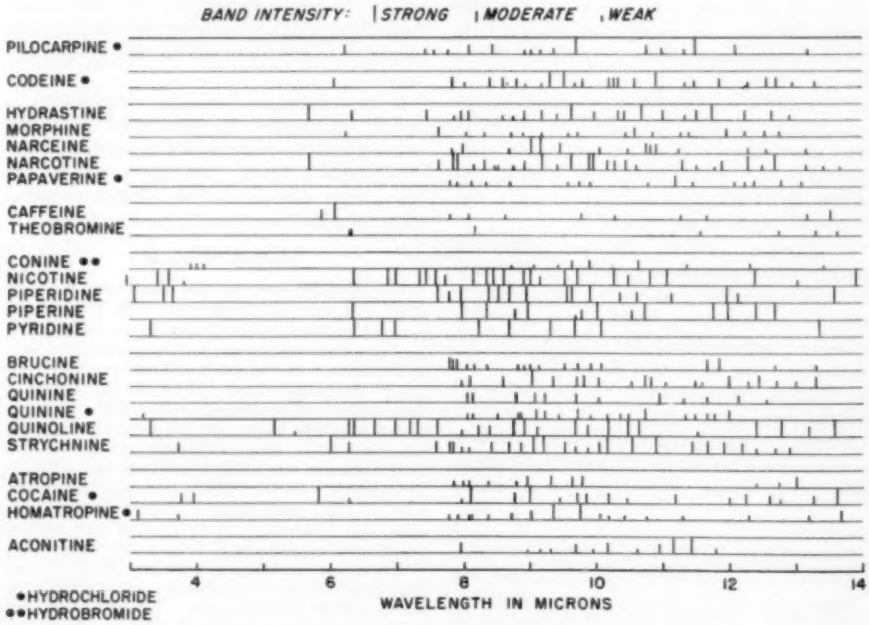

Fig. 1.—Infrared spectra of alkaloids from 3.0 to 14 microns.

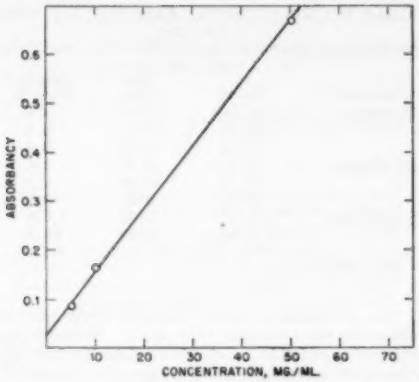

Fig. 2.—Piperine

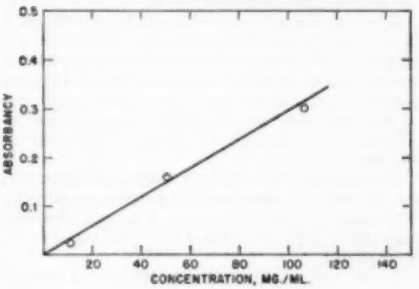

Fig. 3.—Nicotine

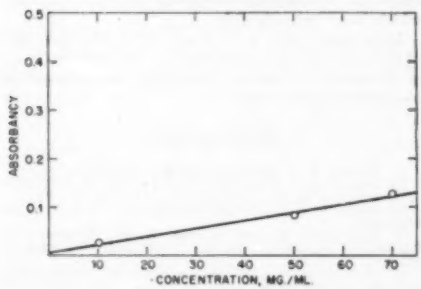

Fig. 4.—Hydrastine

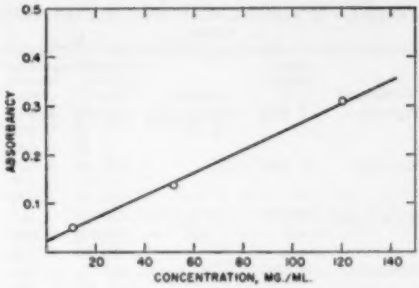

Fig. 5.—Piperidine

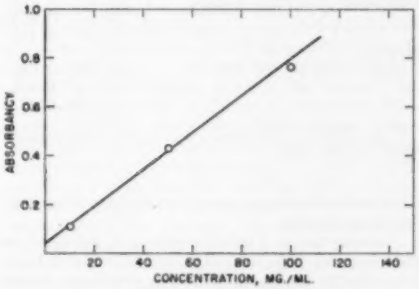

Fig. 6.—Strychnine

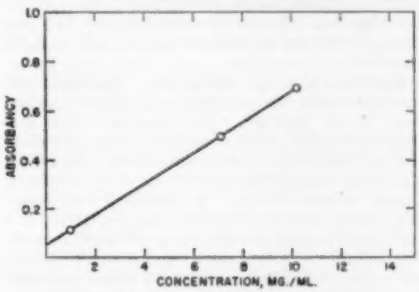

Fig. 7.—Strychnine

Since some of the compounds under investigation were amine salts in the form of the hydrobromide or hydrochloride, quinine and quinine hydrochloride were run to see if the base and its salt have a different spectra. By referring to Fig. 1, it can be seen that the basic amine has fewer absorptions than the amine salt, and although many fall within the same region, the spectra are by no means identical.

To evaluate the method for qualitative analysis, the spectrum of an extract of opium was investigated. Opium in the form of granules was ground in a mortar to a fine powder and extracted with 0.25 *N* HCl. The acid extract was filtered, and the filtrate made alkaline with 1 *N* NaOH. The resulting turbid solution was extracted with ether, then ammonium chloride was added, and a final extraction made with hot chloroform. As a result of this procedure, morphine, narcotine, narceine, and papaverine should be the main constituents extracted (5). A portion of the gelatinous residue from the evaporation of the combined solvent extracts was placed in a 0.025-mm. cell and its spectrum obtained. Of the bands obtained, the following possible assignments could be made; six to morphine, nine to narcotine, three to narceine, and three to papaverine. Five bands in the spectrum were not assignable to any of the alkaloids studied.

Quantitative Estimation.—The first step in quantitative work is the selection of a strong absorption band which is characteristic of the compound under consideration. Figure 1 aids in the selection of a strong distinctive band. Wave lengths in the lower frequency region were preferentially selected,

TABLE I.—QUANTITATIVE ANALYSIS OF PURE ALKALOIDS

Compound	Wave Length, μ	Solvent	Standard Solutions Concn., Mg./Ml.	Absorbancy
Piperine	7.968	$CHCl_3$, CS_2 1:50	50.53	0.673
			10.40	0.165
			5.34	0.085
Nicotine	13.966	CS_2	106.93	0.301
			50.70	0.157
			10.97	0.024
Hydrastine	12.579	$CHCl_3$, CS_2 1:50	70.16	0.126
			50.04	0.081
			10.20	0.025
Piperidine	9.921	CCl_4	120.7	0.307
			51.90	0.136
			10.50	0.049
Strychnine	6.006	$CHCl_3$	100.3	0.779
			50.11	0.424
			10.09	0.110
Strychnine[a]	6.006	$CHCl_3$	10.24	0.695
			7.14	0.446
			1.03	0.117

[a] Cell thickness was increased ten times by use of quarts spacer 1.0 mm. thick.

since these bands are more characteristic of an individual compound.

The selection of a suitable solvent is governed by three factors. The solvent should have high solvent power for the compound under consideration, should not react chemically with it, and, finally, should not show absorption in the wave-length region under consideration. A recently compiled table lists possible solvents for the entire region of 500 to 3,200 $cm.^{-1}$ (6).

Piperine, nicotine, hydrastine, piperidine, and strychnine were used to test the quantitative applications of the method. The standards were prepared by weighing various amounts of the pure alkaloids and dissolving them in the solvent; the resulting solution being made up to the desired volume in a small volumetric flask. A volume of 1 ml. is sufficient when only small amounts are available. For the larger samples volumes up to 10 ml. were used. The solution was well mixed and a small amount withdrawn and added to a fixed cell with a 0.10-mm. spacer. A blank using the same solvents as employed to prepare the standard solutions was used to set the zero absorbancy. Table I shows the solvent, wave length, concentration of the standards, and the absorbancy values obtained.

TABLE II.—QUANTITATIVE ANALYSIS OF PURE ALKALOIDS

Compound	Concn., Mg./Ml. Found	Concn., Mg./Ml. True Value	Relative Error, %
Piperine	14.50	14.00	3.6
	13.30	12.92	0.6
	8.00	7.98	0.3
Strychnine	8.50	8.72	2.6
	6.55	6.46	1.4
	3.60	3.26	9.4
Strychnine[a]	1.11	1.03	7.8
	1.04	0.88	18.2

[a] See Table I.

The absorbancy values obtained were plotted *versus* the concentration (Figs. 2–7). Although the points do not fall in a perfectly straight line in every case, there is a definite trend toward a linear relationship indicating no molecular interaction between the solvent and the alkaloids studied.

To check the accuracy with which unknown solutions could be measured, solutions of piperine and strychnine of known concentration were prepared in concentrations unknown to the operator in the same manner as the standards. The samples were run at the same wave length and the concentration determined from the standard curve. Table II shows the results obtained.

In dealing with relatively high concentrations, i. e., 10 mg./ml., the maximum error is less than 4%. With lower concentrations, i. e., 1 mg./ml., the error may exceed 15% even when a cell thickness of 1 mm. is employed.

In the case of the particular instrument used, it was found necessary to run unknown and standard solutions concurrently since the setting of the slit width is not reproducible.

In practice, once a standard curve has been prepared, only one standard need be run with each set of unknowns so that, if necessary, an appropriate correction may be made.

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The Isolation, Purification and Derivatives of Plant Pigments Related to Rutin*,†

By THOMAS J. HALEY and MURRAY BASSIN‡

A method is described for the extraction and separation of maclurin and morin from *Morus tinctoria*. Comparable information relating to the isolation of iridin from orris root is also reported. Preparation methods for certain derivatives of morin, maclurin, hesperetin, naringenin, and irigenol are described.

RECENT APPLICATION of rutin (3-rhamnoglycoside of 3,5,7,3′,4′-pentahydroxyflavone) in experimental therapy of Roentgen-ray irradiation damage in animals (1, 2) has resulted in a renewed interest in the "vitamin P-like" compounds. Reference to Mayer and Cook (3) reveals that a large number of these compounds of varying chemical structure can be obtained from readily available natural sources. Morin and maclurin have been isolated by extracting with hot water, followed by treatment with concentrated hydrochloric acid [Hlasiwetz and Pfaundler (4), Lowe (5), and Benedict and Hazura (6)], or with lead acetate (7). However, these methods require long time intervals before isolation and purification can be accomplished. Furthermore, the lead acetate method requires an additional treatment to remove the added lead.

We would like to report on a simple hot water extraction procedure which gives an almost quantitative separation of these two plant pigments in a high state of purity, and also on methods of synthesis of derivatives of these and related "vitamin P-like" compounds.

EXPERIMENTAL[1]

Isolation and Purification of Morin (3,5,7,2′,4′-Pentahydroxyflavone).—Three hundred grams of *Morus tinctoria* sawdust was extracted with 24 L. of boiling distilled water until the aqueous extract was almost colorless (about twenty-six hours). This extraction was carried out using 2-L. portions of water and the total extract, after filtration, was concentrated under vacuum at 35° to a final volume of 800 cc. The amorphous yellow powder which precipitated during concentration was removed by centrifugation and dried *in vacuo* over anhydrous $CaCl_2$. This precipitate was extracted with boiling ethanol, filtered, and the filtrate diluted with cold distilled water to precipitate the morin, which was removed by centrifugation and dried over anhydrous $CaCl_2$, yield 1.8 Gm. crude amorphous morin, m. p. 287–288°. Further addition of water and cooling gave an additional yield of 0.6 Gm., m. p. 287–289°. The yield was further increased by adding the washings to the original extract and extracting the resulting solution with ether in a continuous liquid extractor. This latter material, 0.4 Gm., did not melt but sublimed at 289–290°. The total amount of crude morin, 2.8 Gm., was boiled with distilled water, centrifuged, the aqueous layer decanted, and the precipitate dissolved in boiling ethanol. This solution was filtered while hot, and distilled water added to produce clouding. Cooling precipitated the purified morin, which was removed by centrifugation and dried *in vacuo* over P_2O_5. A yield of 2.5 Gm. of silky, almost colorless needles subliming at 289 to 290.5° was obtained. In a sealed tube, morin melted at 303–304° (3). Both the subliming point and the sealed-tube melting point were not depressed when our material was mixed with an authentic sample of pure morin. The solvent purification procedure given above can be eliminated by subliming the crude morin, thus decreasing the losses inherent in solvent purification methods.

Isolation and Purification of Maclurin (2,4,6,3′,4′-Pentahydroxybenzophenone).—The aqueous mother liquor remaining after the removal of the morin was placed in the refrigerator at 2° for seven days. The light yellow precipitate which formed was collected by centrifugation, dissolved in boiling water, filtered while hot, then placed in the refrigerator at 2° overnight to cause precipitation of crude maclurin. This copious precipitate recrystallized from ethanol gave maclurin, faintly yellow needles, m. p. 222 to 222.5° [Mayer and Cook (3) 220–222°], yield 1.45 Gm.

Isolation and Purification of Iridin (7-Glycoside of 5,7,5′-Trihydroxy-6,3′,4′-trimethoxyisoflavone).—Using a modification of Baker's (8) procedure, 1 Kg. of powdered orris root was refluxed with 2 L. of ethanol for thirty minutes, and then filtered while hot. This procedure was repeated three times, and the combined extracts were concentrated to a volume of 300 cc. This solution was kept at a temperature of 5° for three days to precipitate crude iridin as a thick crystalline paste. Three additions of ethanol, 200, 200, and 100 cc.; and one of ether, 100 cc., with stirring for ten minutes after each addition, separated the iridin from all impurities except glucose, which was removed by extraction with 250 cc. of distilled water. The milky white suspension of iridin was filtered, washed with cold water, and dried. A yield of 10 Gm. of iridin, m. p. 208.4 to 208.8°, was obtained [Mayer and Cook (3) 208°].

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‡ The authors wish to thank the American Dyewood Co. for the *Morus tinctoria*, the California Fruit Growers Exchange for the naringin and hesperidin, and Dr. John Bonner of the University of Rochester for the purified morin used in this study.

[1] All melting points given are uncorrected and were determined with an electrically heated Melt-Pointer apparatus, using Anschutz thermometers.

Hydrolysis of Iridin and the Isolation and Purification of Irigenin (5,7,5′-Trihydroxy-6,3′,4′-trimethoxyisoflavone).—Using the method of Baker (8), 6 Gm. of iridin was mixed with a solution containing 1.5 cc. of concentrated H_2SO_4, 30 cc. of ethanol, and 18.5 cc. of distilled water in a sealed tube and heated at 120° for five hours by refluxing the diethyl ether of ethylene glycol around the tube. After cooling, the contents of the tube were poured into a mixture of 30 cc. of ethanol and 20 cc. of distilled water, boiled with charcoal, filtered, 20 cc. of distilled water added, and the solution again treated with charcoal. The filtrate from this solution, upon cooling, deposited almost white rectangular plates of irigenin which were recrystallized from ethanol; m. p. 184.5 to 184.9°, [Baker (8) 185°], yield 2 Gm., 50% of theoretical.

Demethylation of Irigenin and the Isolation and Purification of Irigenol (5,6,7,3′,4′,5′-Hexahydroxyisoflavone).—Using the method of Baker (8), 2 Gm. of irigenin was mixed with 30 cc. of 57% HI (density 1.7) and the solution refluxed for two hours. Waxy crystals separated during the latter part of the reaction, and their yield was increased by the addition of water. After filtration, this material, when recrystallized from 50% aqueous acetic acid, gave irigenol, pale yellow needles, m. p. 330 to 330.5°, dec. [Baker (8) 331°, dec.], yield 1 Gm., 57% theoretical.

Hydrolysis of Hesperidin and the Isolation and Purification of Hesperetin (4′-Methoxy-5,7-3′-trihydroxyflavanone).—Using a modification of the method of Tiemann and Will (9), 3 Gm. of hesperidin was mixed with 20 cc. of 50% aqueous ethanol containing 2% H_2SO_4 and heated in a sealed tube, as described under "Irigenin." Upon cooling, the contents of the tube were poured into distilled water, and the resulting yellow precipitate removed by filtration. This precipitate was dissolved in ethanol, charcoal added, the mixture boiled for two minutes and then filtered. The addition of distilled water and cooling caused the precipitation of hesperetin, pale yellow plates, m. p. 225.5 to 226.5° [Mayer and Cook (3) 226°]; yield 1 Gm., 67% theoretical.

Hydrolysis of Naringin and the Isolation and Purification of Naringenin (5,7,4′-Trihydroxyflavanone).—Using the method of Will (13), 3 Gm. of naringin was placed in a 125-cc. Erlenmeyer flask, 30 cc. of 3% aqueous H_2SO_4 added, and the mixture warmed on a steam bath for ten hours. The oily layer which separated in about three hours gradually became a crystalline solid as the hydrolysis proceeded. This solid was removed by filtration, dissolved in ethanol containing a small amount of charcoal, and boiled for five minutes. After filtration and the addition of distilled water, white crystals of naringenin, m. p. 250.5 to 251°, [Mayer and Cook (3) 251°], precipitated and were collected by filtration; yield 1 Gm., 70% theoretical.

Synthesis of Flavonoid Ethers.—Using the method of Row, Seshadri, and Thiruvengadam (10), 0.5 Gm. of maclurin, 10 Gm. of anhydrous K_2CO_3, and 2 cc. of dimethyl sulfate in 50 cc. of dry acetone were refluxed for forty hours. An additional 0.5 cc. of dimethyl sulfate and 2 Gm. of anhydrous K_2CO_3 were added after sixteen hours. The acetone was removed by distillation, distilled water added to the residue, and the mixture filtered. The resulting white precipitate of pentamethyl maclurin when recrystallized from aqueous ethanol gave colorless plates, m. p. 156.8 to 157.2° [Heilbron (11) 157°]; yield 0.4 Gm., 83% theoretical.

The trimethyl ether of naringenin was prepared in the same manner using 0.3 Gm. of naringenin. Recrystallization from aqueous ethanol gave yellow needles and plates, m. p. 117.2 to 117.8° [Heilbron (11) 117–118°]; yield 0.23 Gm., 66% theoretical.

The pentamethyl ether of morin was prepared in the same manner using 0.5 Gm. of morin. Recrystallization from aqueous ethanol gave white needles, m. p. 156.2 to 157° [Heilbron (11) 155–157°]; yield 0.45 Gm., 73% theoretical.

The dimethyl ether of hesperitin was prepared in the same manner using 0.3 Gm. of hesperetin. Recrystallization from ethanol gave orange platelets, m. p. 153.2 to 154.8° [Tutin and Caton (12) 154°, and Waung (14) 153–155°]; yield 0.1 Gm., 30.5% theoretical.

Synthesis of Irigenol Hexaacetate.—Using the method of Baker (8), 0.3 Gm. of irigenol was boiled for four hours with excess acetic anhydride containing a few drops of pyridine. Recrystallization from acetic anhydride gave colorless prisms of irigenol hexaacetate, m. p. 237.4 to 237.8°, [Baker (8) 237–238°]; yield 0.27 Gm., 50% theoretical.

SUMMARY

1. An improved method for the extraction and separation of morin and maclurin from *Morus tinctoria* has been described.

2. It was observed, contrary to reports in the literature, that morin does not melt at 289 to 290.5°, but sublimes at this temperature. In a sealed tube, the melting point of morin is 303–304°.

3. The hot ethanol extraction procedure for obtaining iridin from orris root yielded better results when the number of extractions was increased from one to three. The increased time required by this procedure was more than compensated for by the larger yield and its high state of purity.

4. The synthesis and total yield of derivatives of morin, maclurin, hesperetin, naringenin, and irigenol have been reported.

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Chromatographic Analysis of Kennewick, Washington Peppermint Oil*,†

By SU MING WANG and HAAKON BANG‡

The region in the vicinity of Kennewick, Wash., produces a high yield of 90 to 100 lb. of peppermint oil per acre. Unfortunately, the oil has an unpleasant odor. The purpose of this investigation was to find the constituent responsible. Several chromatographic methods, adsorptive agents, and eluants were tested. It was found that the constituent responsible for the unpleasant odor could be removed by the adsorption method; however, the constituent was not identified. The adsorption method may be used to obtain menthol from peppermint oil.

THE REGION in the vicinity of Kennewick, Pasco, and Hanford, Wash., produces nearly 100,000 lb. of peppermint oil annually. The yield of oil is reported to be from 90 to 100 lb. per acre, which is among the largest in the United States. Unfortunately, the oil possesses an unpleasant odor which makes it difficult to market. This investigation was carried on in an attempt to isolate, by means of chromatographic adsorption, the constituent which causes the unusual odor.

A complete summary of the composition of American peppermint oil has been made by Power and Kleber (1). They found the following constituents: acetaldehyde, isovaleraldehyde, dimethyl sulfide, amyl alcohol, isovaleric acid, pinene, cineol, phellandrene, limonene, menthone, menthol, menthyl acetate, menthyl isovalerate, lactone, and cadinene.

Foote (2) reported the presence of pulegone associated with *d*-isomethone and small amounts of *d*-neomenthol. He also mentioned the presence of an appreciable amount of 3,6-dimenthylcumarone tetrahydride in Oregon peppermint oil. Tornow and Fischer have made a complete study of the physicochemical properties of peppermint oils from Oregon and Washington (3).

EXPERIMENTAL

The oil used in this investigation was supplied by the I. P. Callison Company of Chehalis, Wash., and was labeled "medium quality Kennewick peppermint oil." The methods described in the United States Pharmacopeia XIII were used in determining menthol, menthyl acetate, and other constants.

The adsorbing agents used in this investigation were activated alumina (Alcoa grade F20, 80–200 mesh), Norit "A," magnesium carbonate, calcium carbonate, and kieselguhr. The first three gave satisfactory results.

The eluants tested were ether, acetone, ethyl alcohol, methyl alcohol, carbon disulfide, and carbon tetrachloride.

Test of Adsorbents.—A glass tube, 40 cm. in length, 0.5 cm. in diameter, was used as a column. This was connected to a suction flask by means of a cork and a small glass tube. A piece of cotton and a small circular filter paper were placed inside the column above the cork as a filter. The adsorbent was packed into the tube to a length of 32 cm., then 100 ml. of a mixture of equal parts of peppermint oil and petroleum ether (b. p. 30–50°) was slowly poured on the top of the column. After the complete addition of the oil mixture, ether was added as an eluant until no odor of menthol remained in the column. The total liquid from the flask was concentrated under vacuum (32 mm. Hg) at room temperature and the constants of the remaining oil determined. Table I shows the results obtained.

Study of Adsorption of Peppermint Oil.—The adsorption column used in the following experiment was 60 cm. long and 3 cm. inside diameter. In order to facilitate the extrusion of the adsorbent, a cylinder of cellophane was placed inside the tube and extending about 5 cm. above the glass tube. Activated alumina was used as an adsorbent.

Fifty cubic centimeters of peppermint oil mixed with the same volume of petroleum ether, reagent grade, was poured into the column and followed by a mixture of petroleum ether and solvent ether in the following proportions: 5 to 3 parts, 3 to 2 parts, and finally equal volumes.

The column was extruded by pouring 5 cc. of petroleum ether between the glass tube and the cellophane. The adsorbent could then be easily removed.

The column was then cut into six sections and the material from each was repacked into columns 25 cm. long and 0.5 cm. i. d. Elution was continued using mixtures of petroleum ether and solvent ether, and, finally, the columns were extruded and cut into three equal sections. The oil from each of these sections was washed from the adsorbent with a mixture of 3 parts of ethyl alcohol and 1 part of ether until no more odor could be detected in the adsorbent. The solvent was removed in a vacuum

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This investigation was supported in part by a grant from the American Foundation on Pharmaceutical Education.
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TABLE I.—CONSTANTS OF PEPPERMINT OIL AFTER PASSING THROUGH ADSORBENT COLUMN

Sample →	Original Oil	Kieselguhr	Calcium Carbonate U. S. P.	Norit "A"	Magnesium Oxide—Heavy 1 pt., Light 1 pt.	Activated Alumina "Alcoa"
Yield, ml.	50	46.5	48	49	48.5	48
Odor	Unpleasant	Unpleasant	Little improved	Pleasant	Pleasant	Pleasant
Sp. gr.	0.9061	0.9078	0.9044	0.9055	0.9057	0.9046
N_d^{25}	1.4651	1.4640	1.4640	1.4643	1.4647	1.4647
$[\alpha]_D^{25}$	−11.72	−12.20	−11.90	−12.52	−12.55	−5.00
Total menthol, %	51.4	50.0	51.1	49.2	50.1	51.1
Total ester, %	6.1	6.02	5.86	3.84	3.95	4.87

at 35 mm. of Hg at room temperature. The oil remaining after the solvent was removed was tested for its refractive index and optical rotation. The oil was then combined for each of the three sections of the last column and tested for total menthol and esters. In this way 18 sections were made. The six sections from the first column were lettered A, B, C, etc., starting from the top. The sections in the second column were numbered 1, 2, and 3, starting from the top. The substance responsible for the unpleasant odor was noticeable in section A_1, but could not be isolated in pure enough form to be identified.

TABLE II.—PROPERTIES OF ELUATES FROM THE EMPIRICAL SECTIONING OF ADSORPTION COLUMN

	Section No.		N_d^{25}	$[\alpha]_D^{25}$	Total Menthol, %	Esters, %
A_1	l[a] 10 cm.		1.4646	−11.2	12.1	4.0
	d[a] 0.5 cm.					
A_2	l 10 cm.					
	d 0.5 cm.		1.4640	− 7.1	12.1	4.0
	l 10 cm.					
A_3	d 0.5 cm.		1.4638	− 8.4	...	...
B_1	l 10 cm.		1.4660	−10.2	...	...
	d 0.5 cm.					
B_2	l 10 cm.		1.4661	−13.1	32.1	1.3
	d 0.5 cm.					
B_3	l 10 cm.		1.4670	−12.1	...	...
	d 0.5 cm.					
C_1	l 10 cm.		1.4660	−12.4	7.8	...
	d 0.5 cm.					
C_2	l 10 cm.		1.4660	−12.4	...	...
	d 0.5 cm.					
C_3	l 10 cm.		1.4660	−11.6	...	...
	d 0.5 cm.					
D_1	l 8 cm.		1.4540	− 7.8	...	...
	d 0.5 cm.					
D_2	l 8 cm.		1.4648	− 6.8	...	...
	d 0.5 cm.					
D_3	l 8 cm.		1.4650	−11.2	...	...
	d 0.5 cm.					
E_1	l 5 cm.		1.4645	− 8.82	...	...
	d 0.5 cm.					
E_2	l 5 cm.		1.4650	−10.1	...	...
	d 0.5 cm.					
E_3	l 5 cm.		1.4648	−11.3	...	...
	d 0.5 cm.		1.4648	−11.3		
F_1	l 5 cm.		1.4670	−11.0	...	...
	d 0.5 cm.					
F_2	l 5 cm.		1.4667	−10.22	...	...
	d 0.5 cm.					
F_3	l 5 cm.		1.4660	−11.2	...	...
	d 0.5 cm.					

[a] l = length of column; d = diameter of column.

The per cent of menthol and esters given in Table II are based on the whole 50 cc. of oil originally used.

Since the menthol content of the oil from the A and B sections of the above experiments was very high, it was decided to try to separate the menthol by chromatography. Magnesium carbonate was used in this experiment because it was more available at the time. Sixty cubic centimeters of Kennewick peppermint oil was added to the column and the oil was eluted with petroleum ether. The column was then extruded and cut into five sections of equal length as before. Each section was lettered A, B, C, etc., starting from the top. These sections were then packed into another column and again eluted with petroleum ether, and, finally, the adsorbent extruded and the column divided into three sections. The sections were labeled A_1, A_2, and A_3, starting from the top as before. The solvent was then allowed to evaporate off spontaneously and the remaining oil was placed in an icebox at 0–5° for fourteen days. The menthol crystals that formed were separated by filtration and, finally, recrystallized from alcohol and weighed.

TABLE III.—MENTHOL OBTAINED FROM SECTIONS OF COLUMN CHROMATOGRAM

Section No.	Oil Obtained from Each Section, Ml.	Menthol Obtained
I-a	3	...
b	3.5	Few crystals
c	4	180 mg.
II-a	5	130 mg.
b	5.4	150 mg.
c	4.0	120 mg.
III-a	4.4	180 mg.
b	4	205 mg.
c	4	Few crystals
IV-a	5	...
b	3	...
c	4.2	...
V-a	4.3	...
b	3	...
c	3	...
Total	58	965 mg.

Table III shows the amount of menthol obtained from each section. Although the total amount of menthol obtained was not great, further study of this method may prove to be satisfactory for obtaining menthol from peppermint oil.

SUMMARY

The unpleasant odor of Kennewick peppermint oil can be removed by chromatographic adsorption, using activated alumina, magnesium carbonate, or Norit.

The best eluants studied were found to be petroleum ether and solvent ether.

The use of the adsorption column indicates that various constituents of the oil may be separated.

The use of adsorption column suggests a method of obtaining menthol from peppermint oil.

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Note

A Note on the Effect of Antihistamine Drugs on Experimental Vomiting*

By CHARLES E. MOSER, JOSEPH W. E. HARRISSON, JULIAN L. AMBRUS, and CHARLES A. LEONARD

RECENTLY the effectiveness of different antihistamines was described against nausea and vomiting of seasickness (1-3), airsickness (4, 5), pregnancy (6), and streptomycin treatment (7). The aim of this investigation was to elicit information as to the location of action of antihistamines on vomiting, to establish a convenient experimental test for this effect, and to determine whether the vomiting inhibitory effect is parallel to antihistaminic activity of the different drugs.

Benadryl, Dramamine, Neo-Antergan, and Phenergan (3277 RP)[1] were tested, these antihistamines being named in order of increasing antihistamine potency, but not necessarily their specificity. Twenty milligrams of these drugs per kilogram of test animal was injected subcutaneously fifteen minutes prior to the administration of the emetics. The following emetics were used in dogs: 0.2 mg./Kg. apomorphine subcutaneously, 25 ml. of a 1% solution of copper sulfate administered by gastric tube. In pigeons digitoxin was used: 0.182 mg./Kg. given intravenously was termed one vomiting dose. Because of the large individual variation in digitoxin sensitivity in some pigeons not responding to a single dose, we had to use two vomiting doses to provoke vomiting. Pigeons requiring higher doses were discarded. For calculation of the dose required, 10% of the weight was deducted for the feathers. All animals which survived were "crossed over," using the control group one week later for the experiment, and the experimental group as the control.

Vomiting was not inhibited by any of the antihistamines under test when they were given prior to the administration of any of the emetics used. This condition applied to both species of animals in the study.

Chen and Ensor (8) experienced a degree of vomiting control in dogs when 0.025 mg./Kg. of apomorphine administered intravenously was preceded by 20 to 40 mg./Kg. of Benadryl given orally. Uniform emetic response was not observed by us with doses of less than 0.2 mg./Kg. of apomorphine given subcutaneously.

During the experiment there was a suggestion by reason of the character of the reaction (increased toxicity) that antihistamine may be synergistic toward digitoxin. Sufficient data are not yet available to confirm this, but such a finding would not be inconsistent with the opinion expressed by Dews and Graham (9) and Reuse (10), who observed a quinidine-like action on the isolated heart by certain antihistamines.

* Received October 13, 1950, from LaWall Memorial Laboratory of Biochemistry and Pharmacology, Philadelphia College of Pharmacy and Science.

[1] Benadryl was kindly supplied by Parke, Davis & Co.; Dramamine by G. D. Searle & Co.; Neo-Antergan and Phenergan by the Rhône Poulenc Co.

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Book Reviews

Handbook of Antibiotics. By A. L. BARON. Reinhold Publishing Corporation, New York, 1950. viii + 303 pp. 15.5 x 23.5 cm. Price $6.50.

This book is designed to meet the needs of anyone requiring information on the methods of production, chemistry, toxicity, bacteriology, and pharmacology of the numerous antibiotic substances which had been isolated and studied prior to the middle of 1949. This information, in general, appears in the form of a running outline, with tables and statistical summaries reduced to the basic facts. The individual antibiotics are treated in alphabetical sequence, which adds to the usefulness of the book as a reference to the over-all status of a particular antibiotic. The author describes the type of data compiled as primarily "basic" information relating more or less directly to the subjects of antibiotics themselves rather than the application in applied and theoretical fields.

The usefulness of the book is enhanced by a chapter on the certification of antibiotics by Henry Welch of the Division of Antibiotics of the Federal Security Agency, Food and Drug Administration, and a chapter by Walter J. Derenberg on the selection and registration of trademarks for antibiotics. A useful table of existing registrations for basic antibiotics, compiled by Mr. Derenberg, Trademark Counsel, of the U. S. Patent Office is also included.

Dr. Baron's book is well documented with basic references to the original literature on each antibiotic discussed and it includes a table of antibiotic-producing organisms, an author index, and an index of organisms mentioned in the text. The book is recommended as a quick source of information on any of the known antibiotics.

Soybeans and Soybean Products. Vol. I. Edited by KLARE S. MARKLEY. Interscience Publishers, Inc., New York, 1950. xvi + 540 pp. Illustrated. 15.5 x 24 cm. Price $11.

Originally the publishers of *Soybeans and Soybean Products* planned a single volume to cover this extensive subject in a comprehensive manner. It soon became apparent, however, that a single volume would be unwieldy, and a decision was reached to publish the work in two volumes of approximately equal size. When one considers the development of the soybean as an agricultural crop between 1925 and the present time, and the new industries created through the technological utilization of this legume, the extent of the present volume and the one contemplated is not surprising.

Soybeans and Soybean Products is the result of a cooperative effort by 26 authors and the editor. Volume I includes 14 of the 25 chapters planned for the book when completed. A notion of the extent of the first volume can perhaps best be conveyed by indicating the coverage of the three sections, the chapters included in each, and the names of the authors contributing one or more chapters.

A. Production.

1. History of Soybean Production (W. J. Morse).
2. World Soybean Production and Trade (E. L. Burtis).

B. Structure and Composition.

3. Structure and Genetic Characteristics of the Soybean (L. F. Williams).
4. Chemical Composition of Soybean Seed (W. J. Morse).
5. Chemical Composition of Soybean Oil (B. F. Daubert).
6. Chemical Characteristics of Soybean Oil (Stewart T. Bauer).
7. Physical Properties of Soybean Oil (M. E. Jefferson).
8. Proteins and Other Nitrogenous Constituents (Sidney J. Circle).
9. Other Constituents of the Soybean (B. F. Daubert).
10. Nutritive Factors in Soybean Products (H. H. Mitchell).

C. Processing.

11. Grading and Evaluation of Soybeans and Derived Products (Egbert B. Freyer).
12. Handling and Storage of Soybeans (Leo E. Holman).
13. Biological Processes in Stored Soybeans (Max Milner).
14. Mechanical Processing of Soybeans (Louis F. Langhurst).

Volume I of *Soybeans and Soybean Products* is well documented and the printing and binding is typical of the usual high standards early established and always maintained by Interscience Publishers, Inc. The pagination will be continuous through the two volumes and an index to both volumes will be included in the second volume, which will be published sometime in the near future.

Pharmacological Basis of Penicillin Therapy. By KARL H. BEYER. Charles C Thomas, Publisher, Springfield, Ill., 1950. xv + 214 pp. 14 x 22.5 cm. Price $4.50.

The purpose of this monograph, according to the author, has been to present briefly the pharmacological basis of penicillin therapy in a concise and reasonably complete form. An examination of the book is convincing proof that Dr. Beyer has accomplished his purpose. In chapters 1 and 2, much of the basic pharmacology pertaining to absorption, distribution, and inactivation of penicillin has been summarized and clearly presented. In chapter 3 the mode of action of penicillin involving the two current philosophies of dosage regime in therapy are discussed. Chapter 4 is devoted entirely to a discussion of the repository penicillin preparations. Chapters 5, 6, and 7 deal with the renal elimination of

penicillin, and measures that have been developed to suppress reversibly its tubular secretion. The pharmacology and clinical utility of combined carinamide and penicillin therapy have been reviewed. Information on Benemid available at the time this book went to press is summarized in an addendum to the text.

The book is well documented with pertinent references at the end of each chapter. The book is provided with an adequate index and the printing and binding are of good quality. The book should be of interest to pharmacologists connected with pharmaceutical institutions.

Plant Viruses and Virus Diseases. 3rd ed. By F. C. BAWDEN. The Chronica Botanica Co., Waltham, Mass.; Stechert-Hafner, Inc., New York City, 1950. xiv + 335 pp. Illustrated. 17 x 27 cm. Price $9.

The study of viruses and virus diseases has a multitude of ramifications into many fields of science. It especially requires that one restudy certain basic principles in chemistry, physics, and biology in order to follow observations which have advanced the subject during the past seven or eight years. Thus, a reader of a textbook on viruses, such as that by Dr. Bawden, finds himself putting together many pieces from theories on quantum mechanics to the property of the gene, from theories of protein chemistry to histological biology, and from epidemiology to entomology, as the present-day knowledge of plant viruses begins to unfold more clearly.

Certainly the very early observations on symptoms of virus infections in plants, as for example in the tulip, tomato, tobacco, and potato, and the association of these with animal infections such as smallpox laid the framework, as Bawden has nicely shown, for great speculations on the subject. These speculations began late in the 19th century and continue even more vigorously today. Among the basic arguments has been that which centers between those who would make the virus a bacterial particle of size difference and those who would place more importance upon more fundamental difference than size relationships. Bawden utilizes two chapters in particular, one on virus crystallinity and the second on particle size, which excite one's speculation into these factors as they support or refute the argument.

The author treats with considerable detail many of the techniques which are conventional for estimating particle sizes in plant virus study, virus purification, assaying, and certain serological reactions. Literature references which are included at the end of each of the 17 chapters are most complete, and therefore of fine value to a researcher in this field. Much of the data in the text has been acquired from Bawden's own observations, as well. At least a third of the book deals with descriptions of virus symptomology in plants, including external and internal signs, and the relationships between viruses and certain of their vectors. Chapter 6, which deals with virus strains, summarizes various effects that are obtainable when plants already infected with one virus are inoculated with known strains from another source. This method of treatment is about as comparable with certain conventional animal immunization techniques as one can imagine. It provides interesting reading and, beyond this, it excites one's imagination as to what mechanisms in a plant can be likened to antibody factors found in the blood of animals.

A treatise on the subject of any disease would be seriously inadequate were control measure or the subject of treatment omitted. Obviously the first reaction for control is to eradicate by totally destroying an infected plant. This does not always aid, however, when infection occurs within an economically important crop or when perennials are to be reckoned with. Nor does it assist enough when unknown plant or animal vectors are likely responsible. Therefore the text surveys rather nicely, in Chapter 16, such aids in virus control as "good farming" and the role of the plant breeder who must develop virus-resistant strains. In returning to the concept of natural immunity as a normal phenomenon in plants, the author finds many possibilities for virus control in the use of plant inoculations such as artificially infecting the organism with avirulent strains. The limiting factors of this method are also wisely touched upon, as are the uses of certain insecticides if the vector be an animal.

An advanced text which is developed from the literature as completely as is this one often becomes loaded with conflicting data, and places the reader in the position of "judging for himself." Nevertheless, the author has organized the material well, and fortunately has not been one-sided to the extent of omitting data, however controversial they may appear. One cannot help but admit the fact that, although great progress has been made in recent years on plant virus studies, there is still no adequate definition for the term "virus" and many of the problems for controlling such infections remain unsolved.

A review of this text would not be complete without some mention of the excellent illustrations which have been included. Not only has the author rewritten and entirely revised his previous two editions, but he has added many new photographs which bring it up to date. These include several excellent electron photomicrographs and ordinary photomicrographs showing crystalline virus structures. All are exceedingly clear and instructive—H. W. YOUNGKEN, JR.

Antihistamines: Industry and Product Survey. By NATHAN WISHNEFSKY. Chemonomics, Inc., New York, 1950. 157 pp. 15.5 x 23 cm. Price $5.

In this book an attempt has been made to present pertinent information relative to the physiological history, the chemical history, and a comparison of the clinical effectiveness of the antihistamine drugs and their usefulness in the treatment of colds. The book also includes an appendix containing tables showing the chemical structure of the important antihistaminics, a list of antihistaminic drugs used experimentally, and a list of antihistamine preparations. The book may serve a useful purpose in its present form, but its interest and usefulness would have been greatly increased if it had been provided with an index and had been more carefully documented in certain instances. The book is printed by an offset method, and it impresses one as having been hastily and carelessly thrown together.

The Dispensatory of the United States of America. 1950 Edition. Vol. 2. New Drug Developments Volume. ARTHUR OSOL, Editor-in-Chief. J. B. Lippincott Company, Philadelphia, 1950. 128 pp. 18 x 27 cm. Price $5.

The United States Dispensatory is a pharmaceutical institution. Its value as a pharmaceutical reference book is learned quite early in the career of every pharmacist. It has undergone twenty-three complete revisions since the appearance of the first edition, published in 1833. The 24th edition was published in 1947 and reviewed [THIS JOURNAL, **37**, 43(1948)] with favorable comments on its form and contents. At the time of publication of the 24th edition, there was some speculation among pharmacists as to what course the *Dispensatory* would follow in keeping up to date as a commentary on the official compendia. We now have the answer to this speculation in Volume 2 of the *United States Dispensatory*.

This work is intended to serve as a companion to the 24th edition. It presents, in the usual style of the *Dispensatory*, the new drug developments of greatest importance which have occurred since 1947. This includes 171 new titles appearing in U. S. P. XIV, N. F. IX, and the British Pharmacopœia 1948. In addition to this coverage, 88 nonofficial drugs are described. Volume 2 of the *Dispensatory* should be considered as Parts Six and Seven to the 24th edition. In Part Six are included monographs on several drugs not previously described in the *Dispensatory*. Among these are such items as aureomycin hydrochloride, chlorampenicol, dihydrostreptomycin, antihemophilic globulin, penicillin G procaine, and vitamin B_{12}. In Part Seven, of especial interest, are the descriptions of 18 new antihistaminic drugs which have been developed and made available during the past three years. In addition, other new drugs, of which cortisone, pregnenolone, ACTH, hyaluronidase, terramycin, and tetraethylpyrophosphate are typical examples, have been fully described.

In Volume 2 of the 24th edition of the *Dispensatory*, the same style and arrangement established for Volume 1 have been followed. As in Volume 1, pertinent references to the original literature have been included. The index is of the same character as that in Volume 1, and the printing and binding are of the same quality. The pagination is continued from the last page of the first volume.

The Dispensatory of the United States of America, 1950 Edition. Vols. 1 and 2. By ARTHUR OSOL and GEORGE E. FARRAR. J. B. Lippincott Co., Philadelphia, 1950. xxii + 2,057 pp. 18.5 x 27 cm. Price $25.

The Dispensatory of the United States of America, 1950 Edition includes Volumes 1 and 2. The two volumes are indexed separately and this edition presents no information not available to owners of the 1947 edition who acquire a copy of Volume 2 bound separately, which has been described briefly in the preceding review.

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BOOKS RECEIVED

Pharmacological Basis of Penicillin Therapy. By KARL H. BEYER. Charles C Thomas, Publisher, Springfield, Ill., 1950. xv + 214 pp. 14 x 22.5 cm. Price $4.50.

Distillation Equilibrium Data. By JU CHIN CHU. Reinhold Publishing Corporation, New York, 1950. v + 304 pp. 15.5 x 23.5 cm. Price $6.

The Dispensatory of the United States of America, 1950 Edition. Vols. 1 and 2. By ARTHUR OSOL and GEORGE E. FARRAR. J. B. Lippincott Company, Philadelphia, 1950. xxii + 2057 pp. 18.5 x 27 cm. Price $25.

The Enzymes—Chemistry and Mechanism of Action. Vol. I, Part I. Edited by JAMES B. SUMNER and KARL MYRBÄCK. Academic Press, Inc., New York, 1950. xvii + 724 pp. Illustrated. 15.5 x 23.5 cm. Price $13.50.

Mycotrophy in Plants. By ARTHUR P. KELLEY. The Chronica Botanica Co., Waltham, Mass.; Stechert-Hafner, Inc., New York City. xiv + 223 pp. Illustrated. 16 x 24 cm. Price $4.50.

Official Methods of Analysis of the Association of Official Agricultural Chemists. 7th ed. HENRY A. LEPPER, Chairman, Editorial Board. Published by the Association of Official Agricultural Chemists, Box 540, Benjamin Franklin Station, Washington 4, D. C., 1950. xv + 910 pp. 16 x 24 cm. Price: U. S. $10.; Foreign, $10.50.

Pharmaceutical Emulsions and Emulsifying Agents By LAWRENCE M. SPALTON. Chemical Publishing Co., Inc., Brooklyn, N. Y., 1950. vii + 132 pp. Illustrated. 14 x 22.5 cm. Price $3.75.

White Mineral Oil and Petrolatum. By ERICH MEYER. Chemical Publishing Co., Inc., Brooklyn, N. Y., 1950. vi + 135 pp. Illustrated. 14 x 22 cm. Price $4.75.

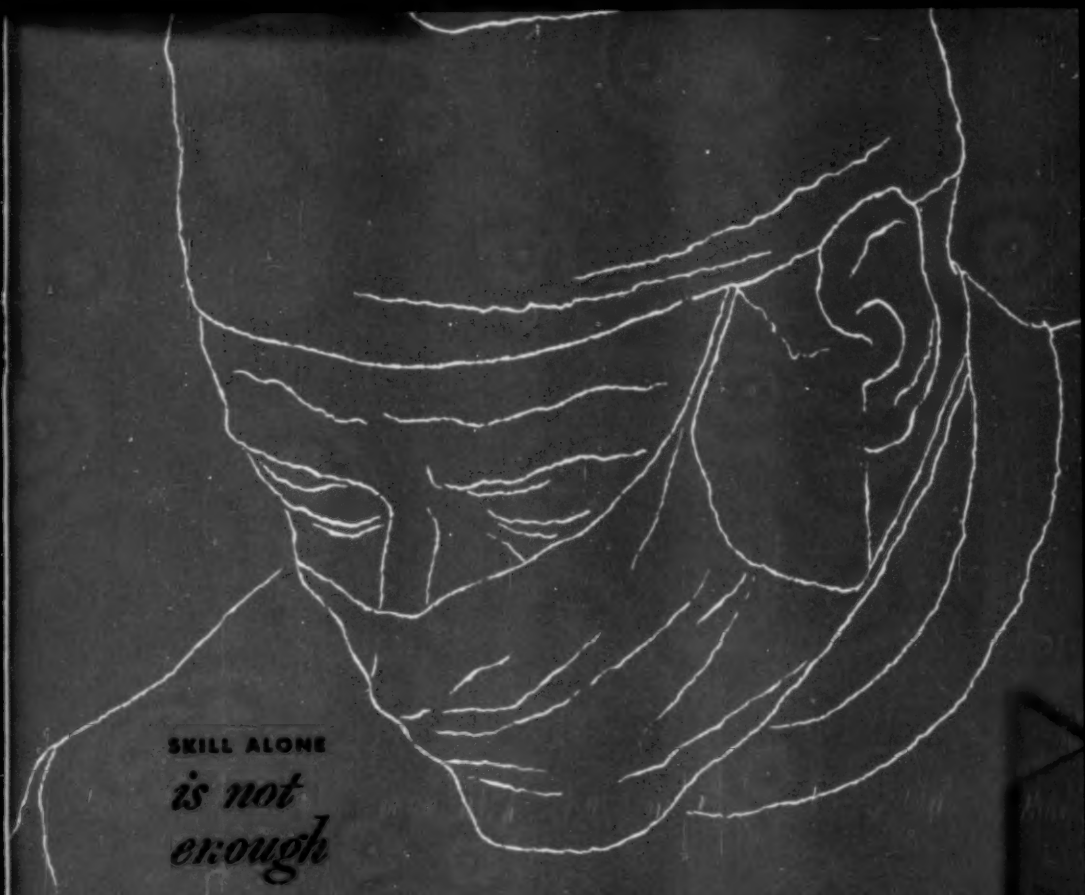
Practical Microscopy. 2nd ed. By L. C. MARTIN and B. K. JOHNSON. Chemical Publishing Co., Inc., Brooklyn, N. Y., 1950. 124 pp. Illustrated. 12.5 x 19 cm. Price $2.50.

Organic Reagents for Organic Analysis. 2nd ed. By the Staff of Hopkin and Williams Research Laboratory. Chemical Publishing Co., Inc., Brooklyn, N. Y., 1950. 263 pp. 14 x 22 cm. Price $5.

Laboratory Manual of Elementary Organic Chemistry. 2nd ed. By GEORGE HOLMES RICHTER. John Wiley and Sons, Inc., New York, 1951. ix + 146 pp. 15.5 x 23.5 cm. Price $1.80.

Pharmacology and Therapeutics. By ARTHUR GROLLMAN. Lea and Febiger, Philadelphia, 1951. 828 pp. Illustrated. 16 x 24 cm.

Heterocyclic Compounds. Vol. 2. Edited by ROBERT C. ELDERFIELD. John Wiley and Sons, Inc., New York, 1951. vii + 571 pp. 15 x 23.5 cm. Price $15.



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